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DIMETHYL SULFOXIDE OXYDATION OF HYDROXY-STEROIDS AND STUDIES ON THE OXIDATION OF THE 5'-HYDROXY GROUP OF NUCLEOSIDES

by

Carmen V. Zenarosa

A Dissertation
Submitted to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Doctor of Philosophy

Western Michigan University
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December, 1969

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PART I

DIMETHYL SULFOXIDE OXIDATION OF HYDROXY-STEROIDS

INTRODUCTION

In our earlier work we demonstrated the usefulness of the acid-catalyzed reaction between N-(p-tolyl)diphenylketenimine (21) and dimethyl sulfoxide (DMSO) for the oxidation of secondary alcohols. This was exemplified by the oxidation of 2',3'-O-isopropylidene-adenosine and other secondary alcohols.[1] We have now extended this procedure to the oxidation of hydroxy-steroids.

Alkynylamines or ynamines can be written in two resonance forms: $R-C\equiv C-\ddot{N}\cdot R'_2$ and $R-\overset{\ominus}{C}=C=\overset{\oplus}{N}R_2$. Because of the close similarity between the latter structure and the structure of a ketenimine ($R_2C=C=N-R'$) and the ability of sulfoxide-ketenimine to effect the oxidation of secondary alcohols, we explored the possibility of using the acid-catalyzed reaction between alkynylamine and DMSO for the oxidation of the hydroxy-steroids. The ynamines N,N-dimethylamino-phenylacetylene and N,N-diethylamino-1-propyne were used for this investigations.

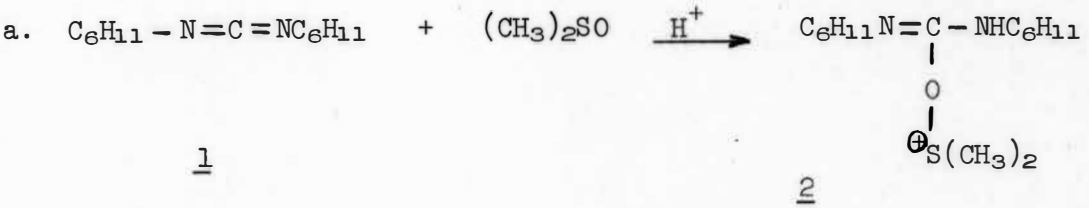
In addition, we have investigated the mechanism of ketenimine-DMSO and ynamine-DMSO oxidations and compared them with the sulfoxide-carbodiimide oxidation.

HISTORICAL

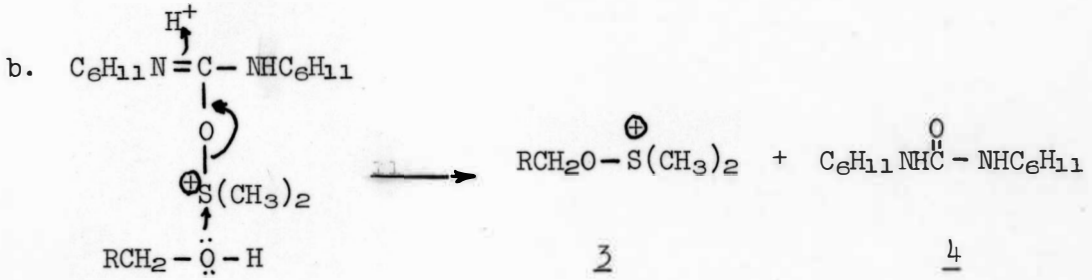
In 1965 Pfitzner and Moffatt[2] reported that the reaction between dicyclohexylcarbodiimide (DCC) and dimethylsulfoxide (DMSO) in the presence of an acid can be used to effect the oxidation of alcohols to the corresponding aldehydes and ketones. Their proposed mechanism for this reaction is outlined in Scheme I. The first step involves the acid catalyzed formation of the sulfoxide-carbodiimide adduct 2. In the second step a nucleophilic attack by the alcohol on the sulfoxonium ion 2 results in the formation of dicyclohexylurea (4) and the alkoxysulfonium ion (3). Finally, abstraction of a proton from the α-carbon of the alkoxy-group in 3 and concerted collapse of the resulting ylid intermediate affords the carbonyl compound and dimethylsulfide. Moffatt et al.[5] substantiated this mechanism by carrying out the oxidation of butanol using $DMSO^{18}$ instead of DMSO. As expected, the resulting butyraldehyde was devoid of any O^{18}-labelled oxygen. In addition, the oxidation of 1,1-dideuteriobutanol led to the formation of 1-deuteriobutyraldehyde and monodeuteriodimethylsulfide. Isolation of the latter compound suggested that proton abstraction from the alkoxy group of 3 is brought about via a cyclic mechanism. The same conclusion was drawn from the formation of pentadeuteriodimethylsulfide from the oxidation of butanol in hexadeuteriodimethylsulfoxide ($DMSO\text{-}d_6$). Finally, oxidation of several O^{18}-labelled alcohols by the DMSO-DCC method led to the formation of O^{18}-labelled aldehydes.[3] These results clearly ruled

out the intermediacy of the pseudoureas shown in Scheme II.

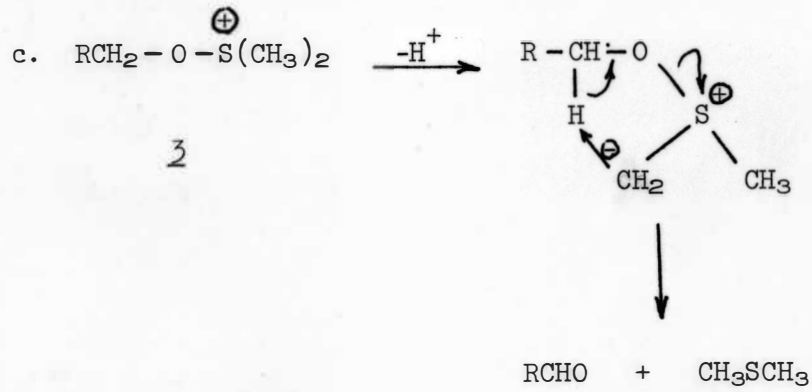
Scheme I

a. 

1 2

b. 

3 4

c. 

3

Scheme II

a. $C_6H_{11}N=C=NC_6H_{11} + RCH_2-O^{18}H \xrightarrow{H^+} C_6H_{11}N=C(-O^{18}H^{\oplus}-CH_2R)-NHC_6H_{11}$

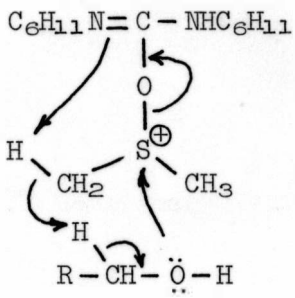
5

b. $C_6H_{11}N=C(-O^{18}H^{\oplus}-CH_2R)-NHC_6H_{11} + (CH_3)_2SO \longrightarrow C_6H_{11}NH-C(=O)-NHC_6H_{11} + RCH_2-O^{18}-S^{\oplus}-(CH_3)_2$

c. $RCH_2-O^{18}-S^{\oplus}(CH_3)_2 \longrightarrow RCHO^{18} + CH_3SCH_3$

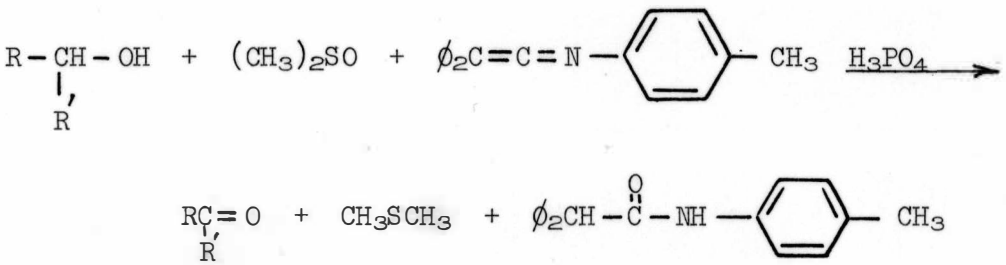
Torssell[4] proposed a concerted mechanism for the DMSO-DCC oxidation. In this mechanism, the first step involves the formation of the sulfoxide-carbodiimide adduct similar to that proposed by Moffatt and Fenselau.[5] The second step consists of a three-body concerted mechanism leading to the formation of dicyclohexylurea, dimethylsulfide, and the corresponding carbonyl compound (Scheme III).

Scheme III



$\longrightarrow C_6H_{11}NH-C(=O)-NHC_6H_{11} + CH_3SCH_3 + RCHO$

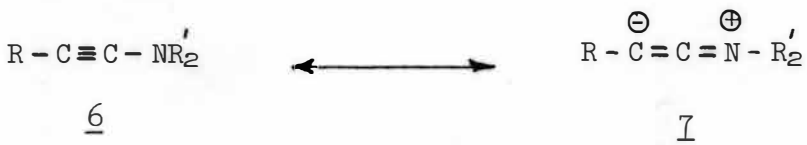
Because of the great similarity between the structures of ketenimines and DCC, N-(p-tolyl)diphenylketenimine was used to oxidize 2',3'-O-isopropylideneadenosine[3] and some secondary alcohols to the corresponding aldehydes and ketones (eq. 1).[1]



(1)

Alkynylamines or ynamines were first prepared by chance in 1958 by N-alkylation of phenothiazine with propargyl bromide. From this reaction, N-(1-propynyl)phenothiazine was obtained instead of the expected isomeric N-(2-propynyl)phenothiazine.[6] However, it was not until 1964 that any additional work was done in this area. Since then a large number of ynamines of the general formula: $R-C\equiv C-NR'_2$ (where R = alkyl-, aryl-, H-, Li-, R_2N-, RS-, halogen, etc.) have been prepared.[7]

As in the case of enamines, the multiple bond and the amino group in ynamines are in conjugation with each other. Both the nitrogen atom and the α-carbon atom can act as sites for nucleophilic attack.

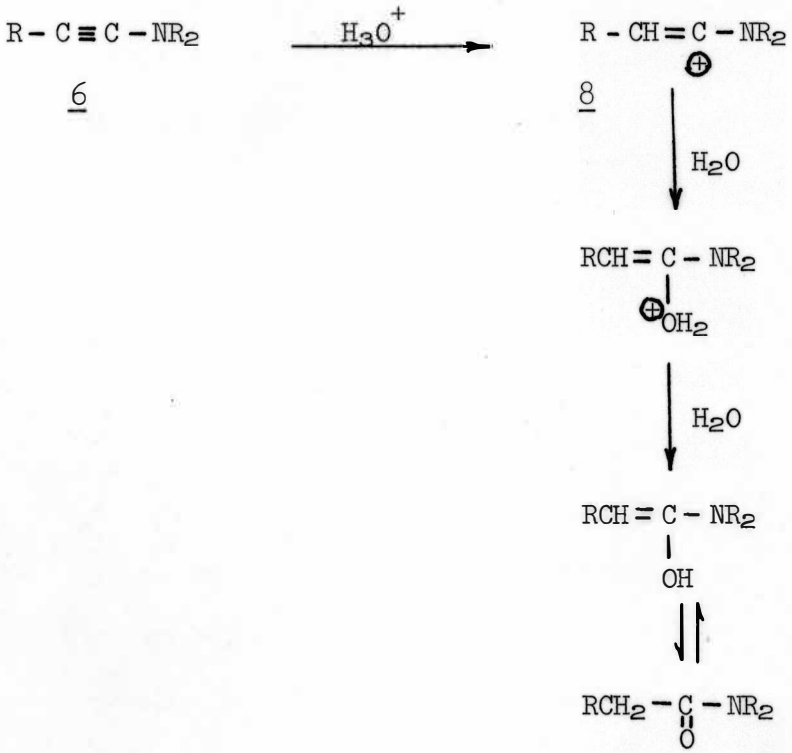


6 I

To date, many interesting reactions have been carried out using these

ynamines. For instance, the exothermic acid-catalyzed addition of water to ynamines affords the corresponding amides (Scheme IV).[8] In addition, ynamines have also been reported to undergo reactions similar to carbodiimide and ketenimines.[7b, 9,8,10]

Scheme IV



EXPERIMENTALS

The melting points were taken on a Thomas-Hoover melting point apparatus and are corrected. A Beckman IR-8 spectrophotometer was used to record the infrared spectra. The nuclear magnetic resonance (nmr) spectra were obtained with a Varian A-60 spectrometer; resonance were measured in ppm downfield from tetramethylsilane standard. Mass spectral data were obtained on an Atlas CH-4 mass spectrometer. Silica Gel G from Brinkman Instruments was used for thin-layer chromatography either on glass slides or 5x20 cm glass plates. Spots on plates were detected either by iodine vapor or by 6 N sulfuric acid spray followed by baking at 100° (ca 15 min). Column chromatography was carried out on a 2.7 x 30 cm glass column packed with chromatography grade silica gel using mixture of chloroform and ethyl acetate as the eluting solvents. Dimethyl sulfoxide (DMSO) was distilled from calcium hydride and stored over Linde Molecular sieves (4a. 1/16) prior to use. Hexadeuteriodimethyl sulfoxide (DMSO-d_6) was also dried over molecular sieves. Pyridine was distilled over phosphorous pentoxide and stored over potassium hydroxide. Petroleum ether (30-60°) was distilled over sodium metal. Alumina was dried at 110° for three days prior to use in the preparation of N-(p-tolyl)diphenylketenimine. N,N-diethylamino-1-propyne, obtained from Fluka AG Chemische Fabrik, was dried over molecular sieves and distilled under reduced pressure.

Oxidation of testosterone using DMSO-d_6 and dicyclohexylcarbodiimide(DCC)

Testosterone (0.64 g, 2.2 mmole) was added, with stirring, to a

solution containing DCC (1, 1.24 g, 4 mmole), dry DMSO-d_6 (1.5 ml), dry benzene (1.4 ml), and 100% orthophosphoric acid (0.2 g, 0.6 mmole). The reaction mixture was stirred at room temperature for 20 hr. The resulting white precipitate (1.1 g) was filtered and washed several times with dry DMSO. Recrystallization of the solid from methanol afforded colorless crystals (0.8 g, 97%), mp 230-231°. A mixture melting point of the crystals with an authentic sample of dicyclohexylurea showed no depression and the infrared spectra (KBr) of the two samples were superimposable (3310 cm^{-1}, NH; 1625 cm^{-1}, C=O). The above filtrate was diluted with benzene, washed with water and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the residue subjected to column chromatography over silica gel. Elution with chloroform-ethyl acetate (4:1) afforded colorless crystals, mp 160-166°. Recrystallization from acetone gave crystals of androst-4-ene-3,17-dione (mp 169-171°) showed no depression and the infrared spectra (KBr) of the two samples were superimposable.

Reaction of DCC with 100% orthophosphoric acid and DMSO-d_6

DCC (1.6 g) was dissolved in dry DMSO-d_6 (1.6 ml) and dry benzene (1.5 ml). Anhydrous orthophosphoric acid (0.8 g) was added with stirring to the resulting solution. After 30 min, the suspension was diluted with benzene (50 ml) and filtered. The resulting precipitate was washed successively with benzene (50 ml), absolute ethanol (50 ml)

and ether. A white crystalline product (0.82 g) was obtained, which after one recrystallization from absolute ethanol gave N,N-dicyclohexylurea (0.16 g), mp 230-231°, lit.[12] 230-231°; ν_{max}^{KBr} (cm^{-1}) 3310 (N-H), 1625 (C=O), no absorption bands around 2475 cm^{-1} due to N-D stretching vibrations were observed.

Preparation of N-(p-tolyl)diphenylacetamide (20)

A mixture of diphenylacetic acid (72 g, 340 mmole) and thionyl chloride (45 ml) was heated on a steam bath for 2 hr. After removal of the excess thionyl chloride, the residue was dissolved in ether and added to a solution containing p-toluidine (39 g, 360 mmole) and triethylamine (26.5 ml) in ether. The suspension was allowed to stand overnight and then filtered. The resulting precipitate was washed four times with ether, dried, and extracted with hot ethyl acetate (600 ml). The insoluble triethylamine hydrochloride was collected by vacuum filtration and washed with petroleum ether (30-60°). Evaporation of the combined filtrate and washings afforded white crystals (84 g). Recrystallization from acetone gave colorless crystals of N-(p-tolyl)diphenylacetamide (75 g, 72.8%), mp 180-182°, lit.[14] mp 180-181°: ν_{max}^{Nujol} (cm^{-1}) 3300 (N-H), 1655 (C=O), 1600 and 1540 (C=C); nmr (DMSO-d_6): δ 2.25 (S, 3, CH_3), 5.21 (S, 1, CH), 7.4 (m, 14, ArH), 10.32 (S, 1, NH).

Preparation of N-(p-tolyl)diphenylketenimine (21)

The procedure of Stevens and Singhal[15] was followed in the preparation of N-(p-tolyl)diphenylketenimine. Alumina (50 g), which had been previously dried at 110° for three days, was added to a solution

of 20 (10 g, 33 mmole) in dry pyridine (300 ml). The above suspension was stirred vigorously while a suspension of phosphorous pentoxide, 25 g in 200 ml of dry pyridine, was added to it. The mixture was refluxed for 7 hr, cooled, and filtered. The precipitate was extracted with dry petroleum ether (30-60°) several times. The combined filtrate and washings were concentrated under reduced pressure. Extraction of the resulting residue with dry petroleum ether gave bright yellow crystals (8.2 g, 87%), mp 79-81°. Recrystallization from petroleum ether afforded 21 (7 g, 74%) mp 82-84°, lit.[14] mp 82-84°; ν_{max}^{Nujol} (cm^{-1}) 2000 (C=C=N).

DMSO-Ketenimine Oxidation of Hydroxy-Steroids

General procedure for DMSO-ketenimine oxidation

The general procedure is exemplified by the oxidation of testosterone. Testosterone (1.35 g, 5 mmole) was added with stirring to a solution of N-(p-tolyl)diphenylketenimine (21, 4.24 g, 20 mmole), dry DMSO (3 ml), dry benzene (3 ml), and 100% H_3PO_4 (0.2 g, 0.6 mmole). The reaction mixture was stirred at room temperature for two days. The progress of the reaction was followed by thin-layer chromatography using chloroform-ethylacetate (4:1) as the developing solvent system. The reaction mixture was diluted with benzene (200 ml) and washed successively with a solution of sodium hydrogen carbonate (10%) and then water, and dried. Evaporation of the solvent afforded a yellow material which upon chromatography on a silica gel column and elution with chloroform-ethylacetate (4:1) gave in succession: N-(p-tolyl)-diphenylacetamide (20), mp 180-182°, lit.[14] mp 180-181°, a small

amount of unidentified material, the desired keto-steroid (1.1 g, 82%), mp 158-161°, and the unreacted hydroxy-steroid, if any. Recrystallization of the keto-steroid afforded colorless crystals of androst-4-ene-3,17-dione (10, 0.9 g), mp 169-171°. A mixture melting point of the crystals with an authentic sample of androst-4-ene-3,17-dione (mp 169-171°) showed no depression, and the infrared spectra (KBr) of the two samples were superimposable: ν_{max}^{KBr} (cm^{-1}) 1715 (C=O, isolated, 1660 (C=O, conjugated).

Unless otherwise mentioned, the reaction procedure and method of isolation are similar for the oxidations of all the hydroxy-steroids used.

Determination of optimum ratio of N-(p-tolyl)diphenylketenimine to testosterone

A series of reactions were conducted using the ketenimine to testosterone ratios given in Table I.

Table I

	N-(p-tolyl)diphenylketenimine		Testosterone	
	No. of mmole	g	No. of mmole	g
1	2.2	0.62	2.2	0.64
2	4.4	1.24	2.2	0.64
3	6.6	1.86	2.2	0.64
4	8.8	2.48	2.2	0.64

The general oxidation procedure was followed using the appropriate amount of the ketenimine, dry DMSO (1.5 ml), dry benzene (1.5 ml), and anhydrous orthophosphoric acid (0.14 g, 0.4 mmole). After the

usual isolation procedure the following yields of androst-5-ene-3,17-dione were obtained: 0.09 g, 15%; 0.19 g, 30%; 0.51 g, 81%; and 0.50 g, 80%, respectively. Mixture melting points of these products with an authentic sample were undepressed.

Oxidation of cholesterol

Cholest-5-ene-3-ol (15, 1.16 g, 3 mmole), which had been previously dried, and N-(p-tolyl)diphenylketenimine (3.45 g, 12 mmole) were dissolved in a mixture of dry DMSO (3.5 ml) and dry benzene (3 ml). After 10 min of stirring, anhydrous orthophosphoric acid (0.2 g, 0.6 mmole) was added and the solution was stirred for two days. The reaction mixture was diluted with benzene and washed first with a solution of sodium hydrogen carbonate (10%), then with water, and dried. The organic layer was concentrated under reduced pressure and chromatographed over a column of silica gel. Elution with chloroform-ethyl-acetate (5:1) provided colorless crystals (0.89 g) which after recrystallization from methanol afforded cholest-5-ene-3-one (16, 0.79 g, 69%), mp 119-120°, lit.[12] 119-120°; ν_{max}^{Nujol} (cm^{-1}) 1725 (C=O, isolated), lit.[12] 1725; $[\alpha]_D^{25}$ -3° (c 2, MeOH), lit.[12] -2° (c 2, MeOH); λ_{max}^{MeOH} 242 mμ, ε 300. Addition of 5 ml of concentrated hydrochloric acid to 1 ml methanolic solution of 16 afforded the isomerized product 17, λ_{max}^{MeOH} 242 mμ, ε 16,600 (see discussion p. 27).

Oxidation of 11α-hydroxyprogesterone (11)

11α-Hydroxyprogesterone (11, 0.99g, 3 mmole), N-(p-tolyl)diphenylketenimine (3.47 g, 12 mmole), dry DMSO (3 ml), 100 % H_3PO_4 (0.2 g, 0.6 mmole), and dry benzene (3 ml) were reacted accord-

ing to the above general procedure. After the work-up, 0.61 g of colorless crystals (62.2%), mp 167-172° were obtained. Recrystallization from methanol afforded pure 11-ketoprogesterone (12, 0.44 g, 45%), mp 172-175°, lit.[16] mp 172-175°. A mixture melting point of the crystals with an authentic sample of 11-ketoprogesterone (mp 172-175°) was undepressed and infrared spectra (Nujol) of the two samples were superimposable.

Oxidation of androst-5-ene-3β,17β-diol

Androst-5-ene-3β,17β-diol (13, 0.87 g, 3 mmole), N-(p-tolyl)diphenylketenimine (3.47 g, 12 mmole), dry DMSO (3 ml), dry benzene (3 ml), and 100% orthophosphoric acid (0.2 g, 0.6 mmole) were reacted according to the general procedure described above. After the usual isolation procedure, colorless crystals (0.54 g, 64%) were obtained. Recrystallization from absolute ethanol afforded androst-5-ene-3,17-dione (14, 0.35 g, 40%), mp 130-146°, lit.[11] mp 130-145°; ν_{max}^{KBr} (cm^{-1}) 1715 (C=O, isolated), lit.[12] 1715; λ_{max}^{MeOH} 240 mμ, ε 210 and on adding 5 ml of concentrated hydrochloric acid to 1 ml of a solution of 14 in methanol gave, after 10 min at room temperature, λ_{max} 240 mμ, ε 16,100 (see discussion on p 27).

Studies on the Mechanism of DMSO-Ketenimine Oxidation

Oxidation of testosterone using DMSO-d_6

The oxidation was carried out in a three-necked round bottom flask (50 ml) connected to an acetone-dry ice (-70°) trap.

Testosterone (0.58 g, 2 mmole), N-(p-tolyl)diphenylketenimine

(1.64 g, 5.8 mmole), dry DMSO-d_6 (1.5 ml), dry benzene (1.5 ml), and 100% anhydrous orthophosphoric acid were reacted following the above general procedure. When the reaction was near completion, the reaction mixture was heated to a temperature of 50° and a small stream of dry nitrogen bubbled through the mixture to facilitate the collection of dimethyl sulfide. Nuclear magnetic resonance analysis of this collected solution showed an absorption at δ2.0 ppm (multiplet), characteristic of CD_3SCD_2H. The dimethyl sulfide was isolated by precipitation with saturated mercuric chloride in absolute ethanol (4 ml). The amorphous product (1.62 g), mp 152-155° was recrystallized from benzene to give colorless crystals of $3HgCl_2 \cdot 2CD_3SCD_2H$ and $3HgCl_2 \cdot 2CD_3SCD_3$ (1.1 g), mp 157-158°, lit.[15] mp 158°. The mass spectrum of this sample had its most intense peak at m/e 67 corresponding to the molecular ion $[CD_3SCD_2H]^+$ and a weak peak at m/e 68 attributed to molecular ion $[CD_3SCD_3]^+$; the ratio of these two peaks was 9:1, which corresponds to about 10% $[CD_3SCD_3]^+$ as compared to $[CD_3SCD_2H]^+$. The other significant peaks were at m/e 50, $[CD_3S]^+$; 200, $[Hg]^+$; 270, $[HgCl_2]^+$.

The yellow-colored residue left after removal of the volatile dimethyl sulfide was subjected to the general procedure used for isolating the keto-steroid. This afforded colorless crystals (1.4 g), mp 175-178°; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 3410 (n-H), 1675 (C=O), and 1595 (C=C). Recrystallization from acetone afforded N-(p-tolyl)diphenylacetamide (1.1 g), mp 181-182°, lit.[14] mp 180-181°. A mixture melting point of this product with an authentic sample (mp 180-181°) showed no depression. Finally, evaporation of the solvent from the final eluate

provided colorless crystals (0.49 g, 78%), mp 156-164°, which upon recrystallization from acetone gave androst-4-ene-3,17-dione (0.39 g), mp 169-171°, lit.[11] mp 169-171°. Infrared spectra of the two samples were superimposable.

Preparation of N-deuterio-N-(p-tolyl)diphenylacetamide (19)

N-(p-tolyl)diphenylacetamide (0.7 g) was suspended in 2 ml of O-deuterioethanol (CH_3CH_2OD). A small amount of hydrogen chloride gas was bubbled through the solution and the resulting suspension was refluxed for 20 min. The solvent was evaporated under reduced pressure. Recrystallization of the resulting material from acetone afforded N-deuterio-N-(p-tolyl)diphenylacetamide (0.6 g), mp 180-181°, lit.[14] mp 180-181°; ν_{max}^{Nujol} (cm^{-1}) 2475 (N-D).

Reaction of N-dueterio-N-(p-tolyl)diphenylacetamide with DMSO-d_6 in the presence of 100% orthophosphoric acid

N-Deuterio-N-(p-tolyl)diphenylacetamide (0.6 g, 2 mmole) and 100% orthophosphoric acid (0.03 g) were dissolved in a mixture of dry DMSO-d_6 (0.5 ml) and dry benzene (0.5 ml). The solution was stirred for two days at room temperature. The resulting mixture was diluted with anhydrous benzene (5 ml) and neutralized with sodium hydrogen carbonate. The suspension was filtered and the filtrate concentrated to give tan-colored crystals which upon recrystallization from acetone afforded N-deuterio-N-(p-tolyl)diphenylacetamide (0.4 g), mp 180-181°, lit.[14] mp 180-181°; ν_{max}^{Nujol} (cm^{-1}) 2475 (N-D).[17]

Reaction of N-(p-tolyl)diphenylketenimine with 100% orthophosphoric acid and DMSO-d_6

A solution of N-(p-tolyl)diphenylketenimine (0.82 g, 1.5 mmole)

in dry DMSO-d_6 (1.5 ml) and dry benzene (1.5 ml) was treated with 100% orthophosphoric acid (0.2 g). The characteristic yellow color of ketenimine disappeared after 30 min. The reaction mixture was heated to 50° and a small stream of dry nitrogen gas was bubbled through the mixture to facilitate the collection of dimethyl sulfide. Nmr analysis of the collected solution showed no absorption. The dimethyl sulfide was isolated by precipitation with saturated mercuric chloride in absolute ethanol (2 ml). The amorphous white powder (0.4 g), mp 152-155°, was recrystallized from acetone to give colorless crystals of $3HgCl_2 \cdot 2CD_3SCD_3$ (0.36 g), mp 157-158°, lit.[16] mp 158°. The solvent from the remaining reaction mixture was evaporated to give a residue which was crystallized from acetone to afford N-(p-tolyl)diphenylacetamide (0.81 g, 90%), mp 180-181°, lit.[14] mp 180-181°. Its infrared spectrum was superimposable with that of an authentic sample of N-(p-tolyl)diphenylacetamide and was devoid of any absorption at 2475 cm^{-1} which is generally attributed to N-D absorption.

Alkynylamine-DMSO Oxidation of Hydroxy-Steroids

Oxidation using N,N-diethylamino-1-propyne

The reaction conditions and isolation procedures were similar for all the oxidation reactions and are exemplified by the oxidation of testosterone. Dry testosterone (0.87 g, 3 mmole) was dissolved in a solution of dry DMSO (3 ml) and dry benzene (3 ml). N,N-Diethylamino-1-propyne (1.66 g, 15 mmole) was added, with stirring, to

the above solution cooled to about 5°. Then 0.2 g of 100% orthophosphoric acid was added to it and the stirring continued. The progress of the reaction was followed by thin-layer chromatography on glass plates coated with Silica Gel G using chloroform-ethyl acetate (4:1) as the developing solvent system. The reaction mixture was poured into ice-water after 30 hr. Filtration of the suspended precipitate afforded a light yellow product (0.85 g). The crude product was dissolved in minimum amount of chloroform-ethyl acetate (4:1) and chromatographed on a silica gel column. Elution with chloroform-ethyl acetate (4:1) gave colorless crystals (0.5 g), mp 136-140°. Recrystallization from methanol gave colorless crystals (0.4 g, 48%) of androst-4-ene-3,17-dione, mp 168-170°, lit.[11] mp 169-170°. A mixture melting point of the product with an authentic sample of androst-4-ene-3,17-dione showed no depression and their infrared spectra (KBr) were superimposable.

Oxidation of cholest-5-ene-3-ol

Cholesterol (1.15 g, 3 mmole), dry DMSO (3 ml), dry benzene (3 ml), N,N-diethylamino-1-propyne (1.66 g, 15 mmole), and 0.2 g of 100% orthophosphoric acid were allowed to react following the general procedure described above. When the reaction was completed as shown by thin-layer chromatography, the reaction mixture was poured into ice-water (300 ml). Filtration of the resulting suspension gave a light yellow precipitate (0.7 g). Chromatography of the crude product using chloroform-ethyl acetate (5:1) as the eluting solvent gave 0.63 g of colorless crystals. Recrystallization from methanol afforded

cholest-5-ene-3-one (0.4 g, 35%), mp 119-121°, lit.[12] mp 119-120°; ν_{max}^{Nujol} (cm^{-1}) 1730 (C=O), lit.[12] 1725 (C=O); λ_{max}^{MeOH} 240 mμ (210) and on adding 5 ml of concentrated hydrochloric acid to 1 ml of the solution in methanol, gave after 10 min at room temperature, λ max 240 mμ (ε 16,060) (see discussion p. 27). A mixture melting point with an authentic sample was undepressed.

Oxidation of 11α-hydroxyprogesterone

11α-Hydroxyprogesterone (0.99 g, 3 mmole), N,N-diethylamino-1-propyne (1.66 g, 15 mmole), dry DMSO (3 ml), dry benzene (3 ml), and 100% orthophosphoric acid (0.2 g) were reacted using the general procedure. After the usual isolation procedure, colorless crystals (0.52 g) of 11-ketoprogesterone were obtained. Recrystallization from methanol afforded pure 12 (0.41 g, 42%), mp 172-175°, lit.[11] mp 172-175°. A mixture melting point of the pure product with an authentic sample of 11-ketoprogesterone (mp 172-175°) was undepressed and infrared spectrum of the two samples were superimposable.

Oxidation of 3β-hydroxyandrost-5-ene-17-one

N,N-Diethylamino-1-propyne (1.66 g, 15 mmole), 3β-hydroxyandrost-5-ene-17-one (0.8 g, 3 mmole), dry DMSO (3 ml), dry benzene (3 ml), and 100% orthophosphoric acid (0.2 g) were reacted as above. Colorless crystals (0.45 g, 60%), mp 126-133°, were isolated after chromatography. Recrystallization from absolute ethanol afforded 0.3 g of androst-5-ene-3,17-dione, mp 130-146°, lit.[12] mp 130-145°; ν_{max}^{KBr} (cm^{-1}) 1730 (C=O); λ_{max}^{MeOH} 240 mμ (ε 208); addition of 5 ml of concentrated hydrochloric acid to 1 ml of this solution gave after 10 min λ max

240 mμ (ε 16,010) (see discussion p. 27).

Oxidation of androst-5-ene-3β,17β-diol

Androst-5-ene-3β,17β-diol (0.87 g, 3 mmole), N,N-diethylamino-1-propyne (1.66 g, 15 mmole), dry benzene (3 ml), dry DMSO (3 ml), and 100% orthophosphoric acid (0.2 g) were reacted according to the above general procedure. White crystals were obtained (0.39 g, 45%) after the typical isolation process. Recrystallization from absolute ethanol afforded androst-5-ene-3,17-dione (0.3 g), mp 130-145°, lit.[12] mp 130-145°. The infrared spectra of both samples were superimposable.

Preparation of N,N-Dimethylaminophenylacetylene

Preparation of 1-chloro-2-phenylacetylene[24]

A solution of phenylacetylene (100 g) in 600 ml of anhydrous ether was added dropwise to an ethereal suspension of sodamide (60 g in 1.15 l of anhydrous ether) in a three-necked flask fitted with a reflux condenser, a mechanical stirrer, and a calcium chloride drying tube. After the addition was completed, the reaction mixture was refluxed for 3 hr or until most of the ammonia had been removed. Then an additional 300 ml of ether was distilled off to ensure complete removal of any residual ammonia. A solution of benzene sulfonyl chloride (180 g) in anhydrous ether (500 ml) was added, with stirring, to the above reaction mixture. When the addition was over, the mixture was further heated under reflux for 4 hr, cooled, and poured into ice-water (1 l). The organic layer was washed thoroughly with cold water (3 x 50 ml) and dried. Distillation under reduced pressure

afforded 80 ml of a slightly impure 1-chloro-2-phenylacetylene, bp 43-45° (ca 1 mm Hg); η_D^{25} 1.5699; ν_{max}^{Neat} (cm^{-1}) 2220 (R-C≡C-R'), lit.[17] 2220.

Preparation of N,N-dimethylaminophenylacetylene (31)[25]

Trimethylamine (21.7 g) and 1-chloro-2-phenylacetylene (15 g) were mixed in a stainless Parr reaction vessel and allowed to react at 55°. The vessel was cooled to room temperature after 40 hr. The semi-solid product was extracted with anhydrous petroleum ether and transferred into a 200 ml round bottom flask. Evaporation of the solvent followed by distillation afforded 7 g of a light brown liquid (90° at ca 40 mm Hg). Redistillation of this fraction gave 31, as a colorless liquid (5 g, 31%), bp 70-71° (ca 1mm Hg); η_D^{25} 1.5849; nmr: δ 2.65 (s, 6, CH_3), 7.25 (m, 5, Ar-H).

Oxidation of Hydroxy-Steroids Using N,N-Dimethylaminophenylacetylene

Oxidation of testosterone

Testosterone (0.44 g, 1.5 mmole), N,N-dimethylaminophenylacetylene (1.5 g, 7.5 mmole), dry DMSO (1.5 ml), dry benzene (1.5 ml), and 100% orthophosphoric acid (0.1 g) were reacted following the procedure described for the oxidation of hydroxy-steroids using N,N-diethylamino-1-propyne. Colorless crystals (0.3 g) were isolated which upon recrystallization from acetone afforded androst-4-ene-3,17-dione (0.26, 61%) mp 169-171°, lit.[11] mp 169-171°. A mixture melting point of the crystalline product with an authentic sample (mp 169-171°) showed no depression. Infrared spectra of these two samples were

superimposable.

Oxidation of 11α-hydroxyprogesterone

N,N-Dimethylaminophenylacetylene (0.49 g, 7.5 mmole), 11α-hydroxyprogesterone (0.49 g, 1.5 mmole), dry DMSO (1.5 ml), dry benzene (1.5 ml), and catalytic amount of 100% orthophosphoric acid (0.1 g) were reacted using similar procedure described for the oxidation of testosterone above. Colorless crystals (0.30 g), mp 151- 160°, were obtained. Recrystallization from methanol gave pure 11-ketoprogesterone (0.22 g, 48%), mp 173-175°, lit. [12] mp 172-175°; ν_{max}^{Nujol} (cm^{-1}) 1730 (C=O, isolated). 1675 (C=O, conjugated). The mixture melting point with an authentic product (mp 172-175°) was undepressed. Infrared spectra of the two samples were superimposable.

Oxidation of 3β-hydroxyandrost-5-ene-17-one

N,N-Dimethylaminophenylacetylene (1.09 g, 1.5 mmole), 3β-hydroxyandrost-5-ene-17-one (0.44 g, 1.5 mmole), dry DMSO (1.5 ml), dry benzene (1.5 ml), and catalytic amount of 100% orthophosphoric acid (0.1 g) were reacted as above. Chromatography on silica gel using chloroform-ethyl acetate (4:1) as the eluting solvent afforded, after evaporation of the solvent, colorless crystals (0.31 g), mp 135-140°. Recrystallization from absolute ethanol provided colorless crystals of androst-5-ene-3,17-dione (0.24 g, 56%), mp 135-145°, lit. [12] mp 130-145°. Infrared spectra of both the materials were superimposable (1715 cm^{-1}, C=O).

Oxidation of androst-5-ene-3β,17β-diol

Androst-5-ene-3β,17β-diol (0.44 g, 1.5 mmole), N,N-dimethylamino-phenylacetylene (1.09 g, 7.5 mmole), dry DMSO (1.5 ml), dry benzene (1.5 ml), and 100% orthophosphoric acid (0.1 g) were reacted and purified according to the general procedure. Chromatography of the crude material (0.41 g) provided colorless crystals of androst-5-ene-3,17-dione (0.18 g, 45%), mp 130-141°, lit. [12] mp 130-145°. Infrared spectrum of this product was superimposable with that of an authentic sample (1715 cm^{-1}, C=O).

Studies on the Mechanism of Alkynylamine-DMSO Oxidation

The oxidation of testosterone using N,N-diethylamino-1-propyne and DMSO-d_6 was repeated following the general procedure described previously, using exactly the same amount of reactants. The apparatus used was similar to that described for the studies on ketenimine-DMSO oxidation mechanism. The solution of CD_3SCD_2H in benzene was collected in the usual manner. The nmr spectrum of this solution (at 10°) showed a multiplet at δ 1.88 ppm, characteristic of CD_3SCD_2H. Addition of saturated mercuric chloride in absolute ethanol (4 ml) gave an amorphous white precipitate (1.3 g), mp 152-156°. Crystallization from benzene afforded colorless crystals of $3HgCl_2 \cdot 2CD_3SCD_2H$ and $3HgCl_2 \cdot 2CD_3SCD_3$ (1 g), mp 157-158°, lit. [16] mp 158°. The mass spectrum of this sample had its most intense peak at m/e 67 corresponding to the molecular ion $[CD_3SCD_2H]^+$ and a weak peak at m/e 68

attributed to the molecular ion $[CD_3SCD_3]^+$. These two peaks were in a ratio of 9:1. Other intense peaks were at m/e 49, $[CD_2SH]^+$; 50, $[CD_3S]^+$; 200, $[Hg]^+$; 270, $[HgCl_2]^+$.

Distillation of the remaining mixture at reduced pressure (1 mmHg) gave a colorless liquid: first fraction (0.32 g), bp 44° and second fraction (1.6 g), bp 45-46°; nmr ($CDCl_3$): δ 1.6 (m, 8.8, $C\underline{H}_3$), 2.32 (q, 2, $C\underline{H}_2$-C(=O)), 3.38 (q, 4, $C\underline{H}_2$-N). Chromatography of the crude residue over silica gel and elution with chloroform-ethyl acetate (4:1) gave androst-4-ene-3,17-dione (0.39 g, 45.8%), mp 169-171°. A mixture melting point with an authentic sample (mp 169-171°) showed no depression.

A similar reaction was conducted using dry DMSO instead of DMSO-d_6. Nuclear magnetic resonance spectrum of the distillate (1.6 g) showed: δ 1.6 (m, $C\underline{H}_3$) and 2.64 (s, CH_3-S) with the ratio of 49:6, respectively. From this ratio it appears that the reaction afforded 1.36 g of N,N-diethylpropionamide. Androst-4-ene-3,17-dione (0.4 g, 46%) was also isolated.

Reaction of N,N-diethylamino-1-propyne with 100% orthophosphoric acid and DMSO-d_6

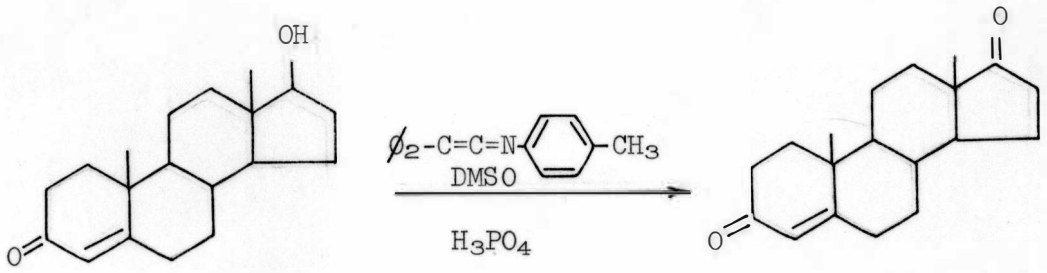
N,N-Diethylamino-1-propyne (1.66 g, 15 mmole) was dissolved in a mixture of dry benzene (1.5 ml) and dry DMSO (1.5 ml). The solution was cooled to ca 5° and then treated with 100% orthophosphoric acid. When the reaction was completed (2 hr) as indicated by the disappearance of the peak at 2200 cm^{-1} (C≡C) in the ir spectrum. The flask was connected to a dry-ice—acetone trap (-70°) and heated to 50° for an additional 40 min with a small amount of nitrogen bubbling

through the solution to facilitate the collection of dimethyl sulfide. Nuclear magnetic resonance spectrum of this solution showed no absorption. The dimethyl sulfide was precipitated with a saturated solution of mercuric chloride in absolute ethanol (2 ml) to give a white amorphous precipitate (0.45 g), mp 152-156° which when crystallized from benzene, afforded colorless crystals of $3HgCl_2 \cdot 2CD_3SCD_3$ (0.39 g), mp 157-158°, lit.[16] mp 158°.

Evaporation of the solvent from the residue and subsequent vacuum distillation (1 mmHg) gave two fractions: first fraction (0.31 g), bp 44°; second fraction (1.61 g), bp 45-46°. Nuclear magnetic resonance spectrum showed: δ 1.6 (m, 9, $C\underline{H}_3$), 2.32 (q, 2, $C\underline{H}_2$-C(=O)), 3.38 (q, 4, $C\underline{H}_2$-N).

DISCUSSION

As an extension of our previous work on the dimethylsulfoxide (DMSO)-ketenimine (21) oxidation of alcohols to the corresponding aldehydes and ketones, we investigated the usefulness of this reagent in the oxidation of hydroxy-steroids. The oxidations were performed in dry DMSO-benzene solutions. During these reactions the exclusion of moisture was imperative due to a competing reaction leading to the formation of N-(p-tolyl)-α-hydroxydiphenylacetamide (25). Using this procedure four hydroxy-steroids were oxidized under very mild conditions (room temperature and 12-48 hr). The progress of the reaction was followed by thin-layer chromatography on glass plates coated with Silica Gel G using chloroform-ethylacetate (4:1) as the developing solvent system. Considering the oxidation of testosterone (9) to androst-4-ene-3,17-dione (10) as a model reaction, it was shown that the best results were obtained by treating the alcohol

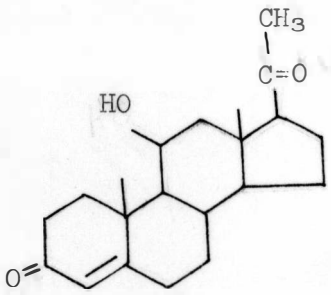


9 10

(one equivalent) with the ketenimine (three equivalents) in anhydrous DMSO or a mixture of DMSO and some suitable inert solvent such as benzene. In all the cases, the products were isolated by column chromatography on silica gel using various mixtures of chloroform-ethylacetate for elution. The result of these oxidations are shown in Table II

Table II

Reactant	Product	Yield (%)
testosterone (9)	androst-4-ene-3,17-dione (10)	82
11α-hydroxy-progesterone (11)	11-ketoprogesterone (12)	62
androst-5-ene-3β,17β-diol (13)	androst-5-ene-3,17-dione (14)	60
cholest-5-ene-3β-ol (15)	cholest-5-ene-3-one (16)	69



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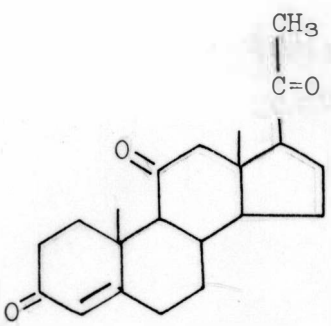

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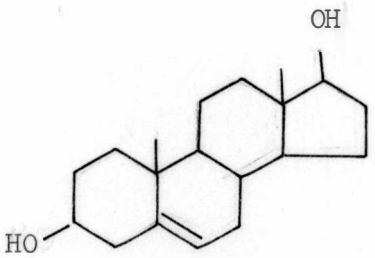
One particularly useful application of this method is in the oxidation of Δ^5-3-hydroxy-steroids as illustrated by the oxidation of cholesterol (15). The most frequently used method of oxidation of



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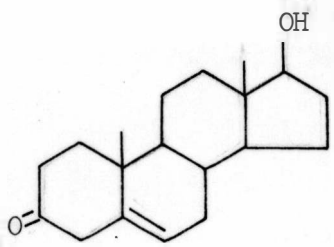
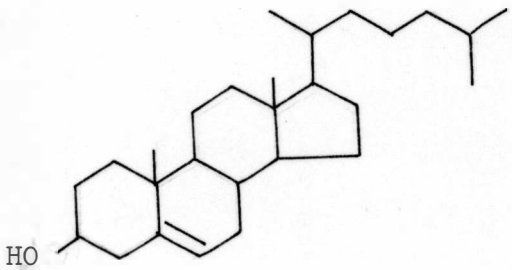


14

hydroxy-steroids is the rapid titration of the alcohol with chromic acid in acetone.[18] However, this leads to isomerization of the carbon-carbon double bond from Δ^5 to Δ^4 position. Using the oxidation procedure involving sulfoxide-ketenimine, only a trace amount of the isomerized ketone 17 was obtained. This was indicated by the absence of an absorbance band at 1670 cm^{-1} (conjugated C=0) in the infrared spectrum and by ultraviolet data before and after the addition of acid (HCl). The keto-steroid (16) showed only the beginning of an end absorption at 240 mμ with ϵ^{MeOH}_{240} 300. Addition of a trace amount of concentrated hydrochloric acid to 16 led to complete isomerization of the Δ^5 double bond (within 10 min) giving the Δ^4-3-ketone 17 (ϵ^{MeOH}_{240} 16,600).



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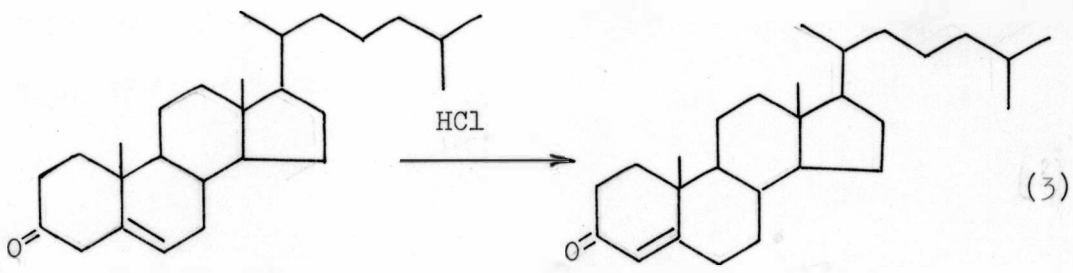
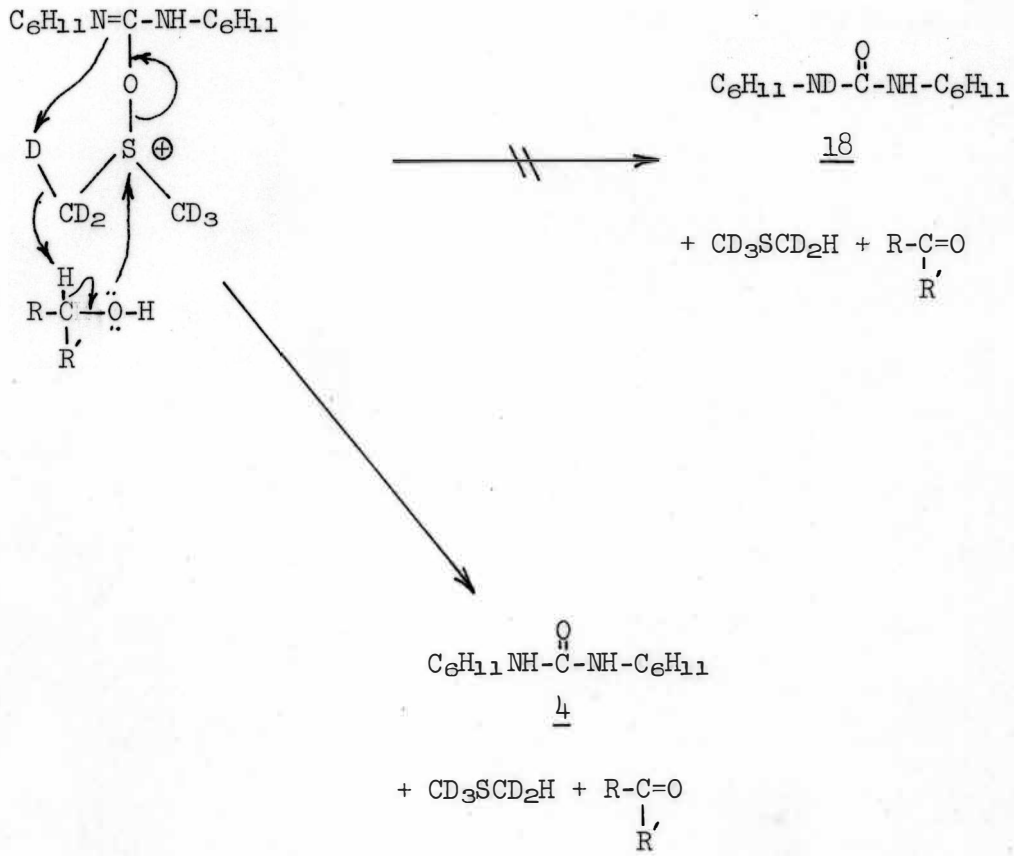


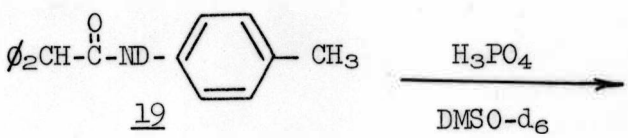
16 17 (3)

As mentioned earlier in Schemes I and III (pp. 3-4), two mechanisms have been proposed for the DMSO-DCC oxidation. Therefore, before investigating the mechanism of ketenimine-DMSO oxidation, we first attempted to test the validity of these two mechanisms. Testosterone was oxidized using hexadeuteriodimethylsulfoxide ($DMSO\text{-}d_6$) and cyclohexylcarbodiimide (DCC) in the presence of catalytic amount of orthophosphoric acid (H_3PO_4). If Torssell's proposed mechanism (Scheme III) were operative, the resulting dicyclohexylurea (18) should contain deuterium (N-D) as shown in Scheme V. However, the infrared spectrum of the dicyclohexylurea (4) isolated from the above reactions indicated the absence of any N-D absorption band around 2475 cm^{-1}. Furthermore, treatment of DCC with $DMSO\text{-}d_6$ in the presence of anhydrous orthophosphoric acid for 30 min also afforded 4 (in quantitative yield) which was devoid of any N-D stretching vibration in the infrared region. The possibility of 18 undergoing a proton exchange to afford 4 was eliminated because dicyclohexylurea is very insoluble (it precipitates out from the solution as it forms) and also by the fact that N-deuterio-N-(p-tolyl)diphenylacetamide (19) did not undergo any proton exchange under identical reaction condi-

tions (eq 4). These results unequivocally ruled out the mechanism proposed by Torsssell (Scheme III) and further substantiated Moffatt et al.'s proposed mechanism (Scheme I).

Scheme V

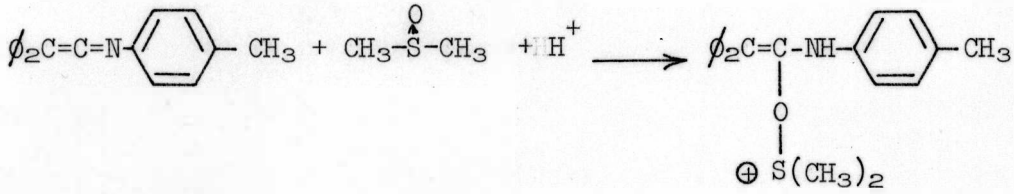




(4)

The oxidation of alcohols using the acid-catalyzed reaction between the ketenimine (21) and DMSO can be explained by the mechanism given in Scheme VI [19]. The sulfoxonium ion as an intermediate (22)

Scheme VI

a. 

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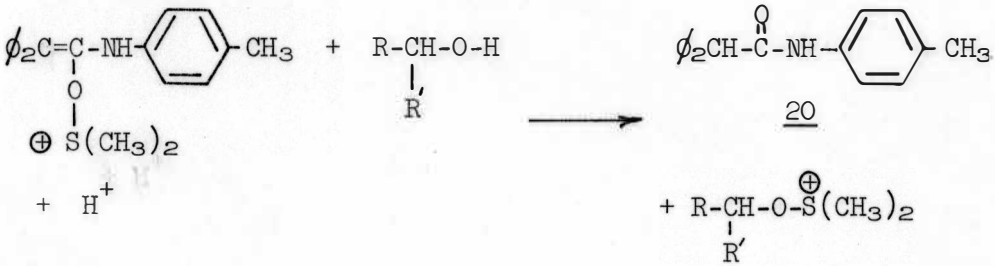
b. 

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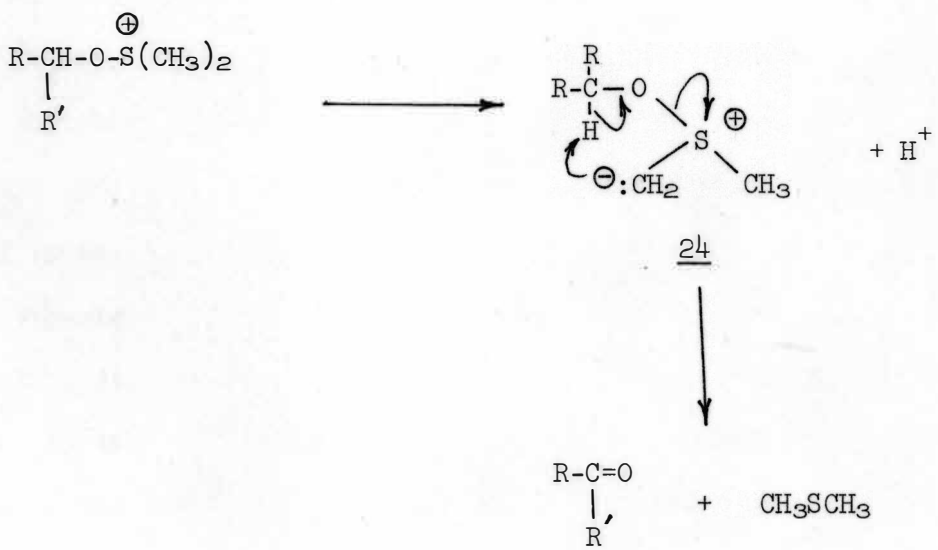
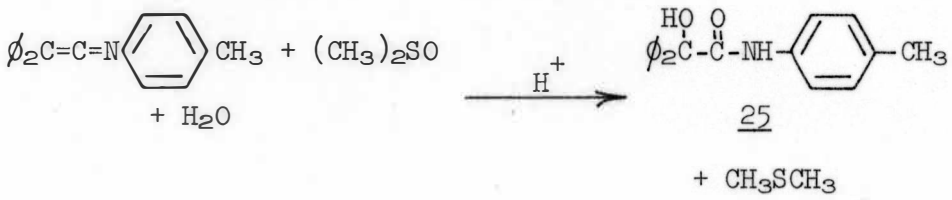
23

c. 

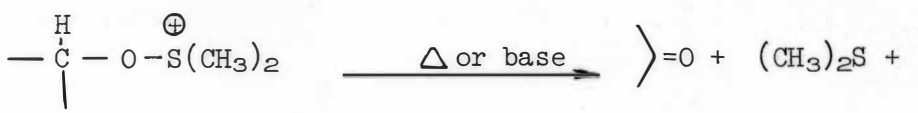
24

mechanistically relates this reaction to the DMSO oxidation of various reactive alkyl tosylates and alkyl halides to carbonyl compounds.[20] The initial formation of the DMSO-ketenimine adduct (22) was proposed by Lillien [16] and is similar to the DMSO-DCC adduct proposed by Moffatt et al.[5] Furthermore, treatment of the ketenimine 21 with DMSO in the presence of concentrated orthophosphoric acid afforded 25 (eq 5), suggesting that in the first step (step a), the protonation takes place on the nitrogen atom rather than on the carbon atom. The



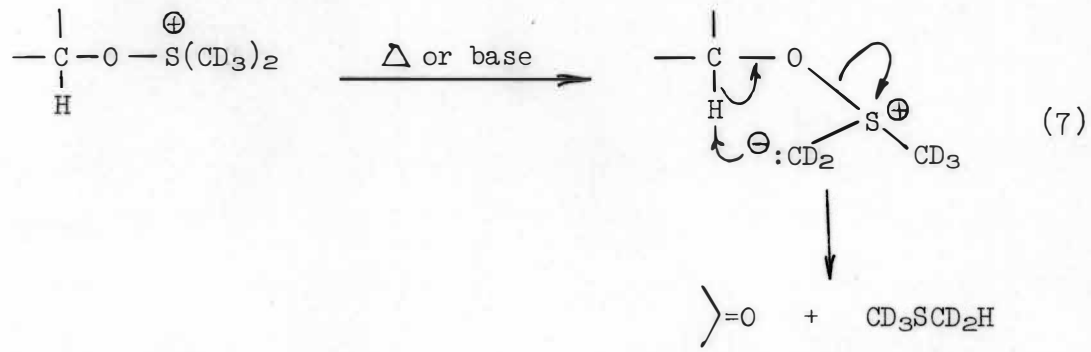
(5)

second step involves the protonation and nucleophilic attack by the alcohol molecule on the sulfoxonium ion 22 resulting in the formation of N-(p-tolyl)diphenylacetamide and alkoxysulfonium ion (23), this step may proceed stepwise or simultaneously. Such an attack is analogous to the known hydrolysis[20,21] and alcoholysis [22] of alkoxysulfonium salts by an S_N2 displacement. In the final step (step c), a proton is abstracted from the S-CH_3 part of 23 and concerted collapse of the resulting intermediate affords the corbonyl compound and dimethyl sulfide. In the case of the DMSO-DCC oxidation an alternative pathway by which the same final products could be formed (eq 6) was previous-

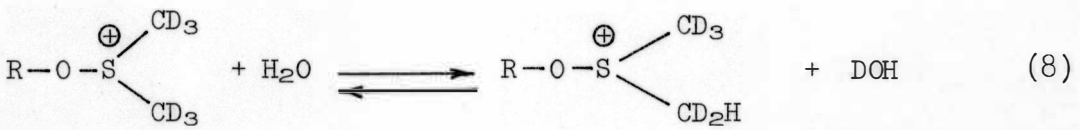


(6)

ly eliminated by Torssell.[4] Provided only a negligeable amount of scrambling occurs in the sulfonium ion 23, before decomposition, the mechanism shown in equation 6 gives rise to hexadeuteriodimethyl sulfide (CD_3SCD_3). On the other hand, the mechanism shown in equation 7 provides pentadeuteriodimethyl sulfide CD_3SCD_2H). Since the results of his experiments indicated the formation of CD_3SCD_2H, equation 6 was ruled out. However, the latter compound can also arise via a hydrogen exchange reaction (eq 8). Recently, Torssell [4] reported

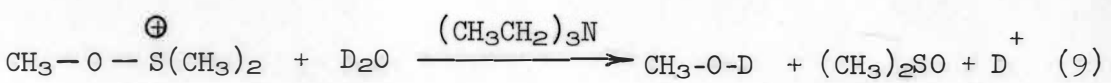


(7)



(8)

that the above reaction (eq 7) is not valid, based on the results obtained when 27 was treated with ten molar excess of deuterium oxide in the presence of catalytic amount of trimethylamine (eq 9). This



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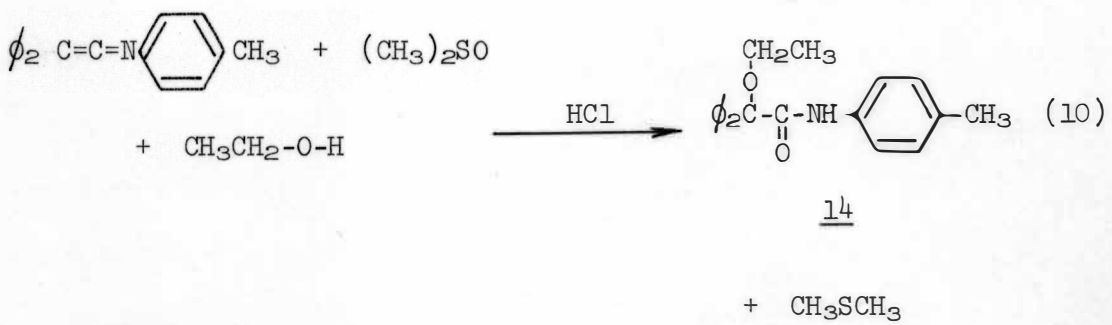
(9)

reaction afforded no deuterium-labeled dimethyl sulfoxide indicating that the hydrolysis (eq 9) proceeds faster than proton exchange.

Since the oxidation was carried out in the presence of free acid (H_3PO_4), the bimolecular abstraction of such a proton is difficult to understand. On the other hand, proton of methyl groups directly attached to positively charged sulfur atom are known to readily undergo exchange with D_2O and the rate of exchange is several hundred times higher in the case of alkoxysulfonium derivatives.[20] Hence, under the very slightly acidic reaction conditions, it is reasonable to assume that proton abstraction promoted by the second dissociation of H_3PO_4 will lead to the d-orbital-stabilized ylid 24. The above reasoning was further supported by the observation that when absolute ethanol was treated with a mixture of ketenimine, DMSO, and anhydrous hydrogen chloride gas, no oxidation occurred. This reaction afforded the α-alkoxy derivative 14 (eq 10).[16]



(10)

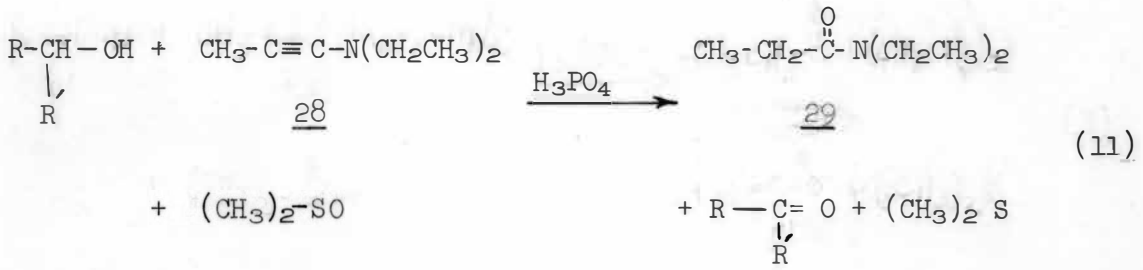
The carbanion in 24 can then act as an internal base. The collapse of 24 via a cyclic mechanism as shown in Scheme VI (step c) gives the carbonyl compound and dimethyl sulfide. Additional evidence supporting the intramolecular proton abstraction, as described above, was provided by oxidizing testosterone in the presence of DMSO-d_6,

N-(p-tolyl)diphenylketenimine, and anhydrous orthophosphoric acid. The nmr spectrum at 10° of the dimethylsulfide-benzene solution isolated from this reaction showed a multiplet at δ 1.88, which can be attributed to CD_3SCD_2H.[4] Furthermore, pure dimethylsulfide was also isolated as a mercuric chloride complex ($3HgCl_2 \cdot 2CD_3SCD_2H$) whose mass spectrum showed an intense peak at m/e 67 due to the molecular ion $[CD_3SCD_2H]^+$ and a weak peak at m/e 68 (relative peak intensity 10%), attributed to the molecular ion $[CD_3SCD_3]^+$. The other intense peaks in the spectrum were: m/e 49, $[CD_2HS]^+$; 50, $[CD_3S]^+$; 200, $[Hg]^+$; 270, $[HgCl_2]^+$. This mass spectral data indicates that the above mercuric chloride complex contains 90% of CD_3SCD_2H and 10% of CD_3SCD_3. Apparently, the CD_3SCD_3 contaminant in CD_3SCD_2H arose from the direct conversion of the excess ketenimine to the amide. This was also supported by the fact that when N-(p-tolyl)diphenylketenimine was treated with DMSO-d_6 in the presence of orthophosphoric acid, the resulting dimethylsulfide was pure CD_3SCD_3 as indicated by its nmr spectrum. The N-(p-tolyl)diphenylacetamide isolated from this reaction proved by nmr and ir spectroscopy to be devoid of any C-D absorption. This observation substantiated the stepwise (Scheme VI) mechanism and refuted an alternative mechanism (Scheme VII) similar to that suggested by Torssell for DCC-DMSO oxidation.

Ynamines have been reported to undergo reactions similar to carbodiimide and ketenimines and in some cases, they have been suggested to proceed via the keteniminium ion 8 ($R\text{-}\overset{H}{\underset{|}{C}}=C\text{-}N^{\oplus}R_2'$) as shown in Scheme IV (p. 6). These observations prompted us to investigate the possible application of the acid catalyzed reaction between ynamines and DMSO

in the oxidation of the hydroxy-steroids.

Oxidation of hydroxy-steroids (one molar equivalent) was conducted in dry DMSO-benzene solution in the presence of N,N-diethylamino-1-propyne (five molar equivalent) and catalytic amount of 100% orthophosphoric acid. It was necessary to cool the reaction mixture to 0° to prevent polymerization of the ynamine. The progress of the reaction was followed by thin-layer chromatography using chloroform-ethylacetate (4:1) as the developing solvent system. In all the cases, the products were isolated by precipitation with water followed by column chromatography using silica gel. Using this procedure five hydroxy-steroids were oxidized giving 45-60% yields of the corresponding keto-steroids (eq 11).[23] The results are shown in Table II.

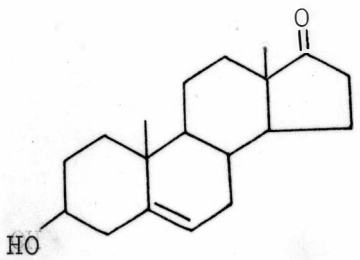


(11)

There was no observed isomerization of a Δ^5-3-ketone to a Δ^4-3-ketone as indicated by the infrared spectrum (absence of a band at 1630 cm^{-1} due to conjugated C=O). In addition, the product obtained showed only the beginning of an end absorption at 240mμ with ϵ_{240}^{MeOH} (16,000). Oxidation of hydroxy-steroids using N,N-dimethylaminophenylacetylene (31, eq 12) afforded the corresponding keto-steroids in relatively better yields (Table III). The results seemed reasonable, for if we consider the resonance reference structure of I ($R-\overset{\ominus}{C}=C=\overset{\oplus}{N}-R_2$),

Table III

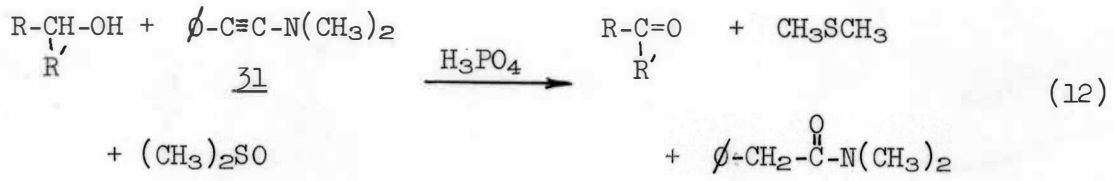
Reactant	Product	Yield (%)
testosterone (9)	androst-4-ene-3,17-dione (10)	60
11α-hydroxy-progesterone (11)	11-ketoprogesterone (12)	53
cholest-5-ene-3β-ol (15)	cholest-5-ene-3-one (16)	55
3β-hydroxyandrost-5-ene-17-one (30)	androst-5-ene-3,17-dione (14)	60
androst-5-ene-3β,17β-diol (13)	androst-5-ene-3,17-diol (14)	45



30

N,N-diethylamino-1-propyne (28) contains an electron releasing group (R= CH_3) which tends to destabilize this particular structure by inductive effect. On the other hand, this structure is further stabilized when the methyl group is replaced by a phenyl group as in the case of N,N-diphenylaminophenylacetylene (31).

Our proposed mechanism for ynamine-DMSO oxidation is outlined in Scheme VIII. It is similar to the mechanism proposed for DCC-DMSO and ketenimine-DMSO oxidations. The first step in the proposed mecha-



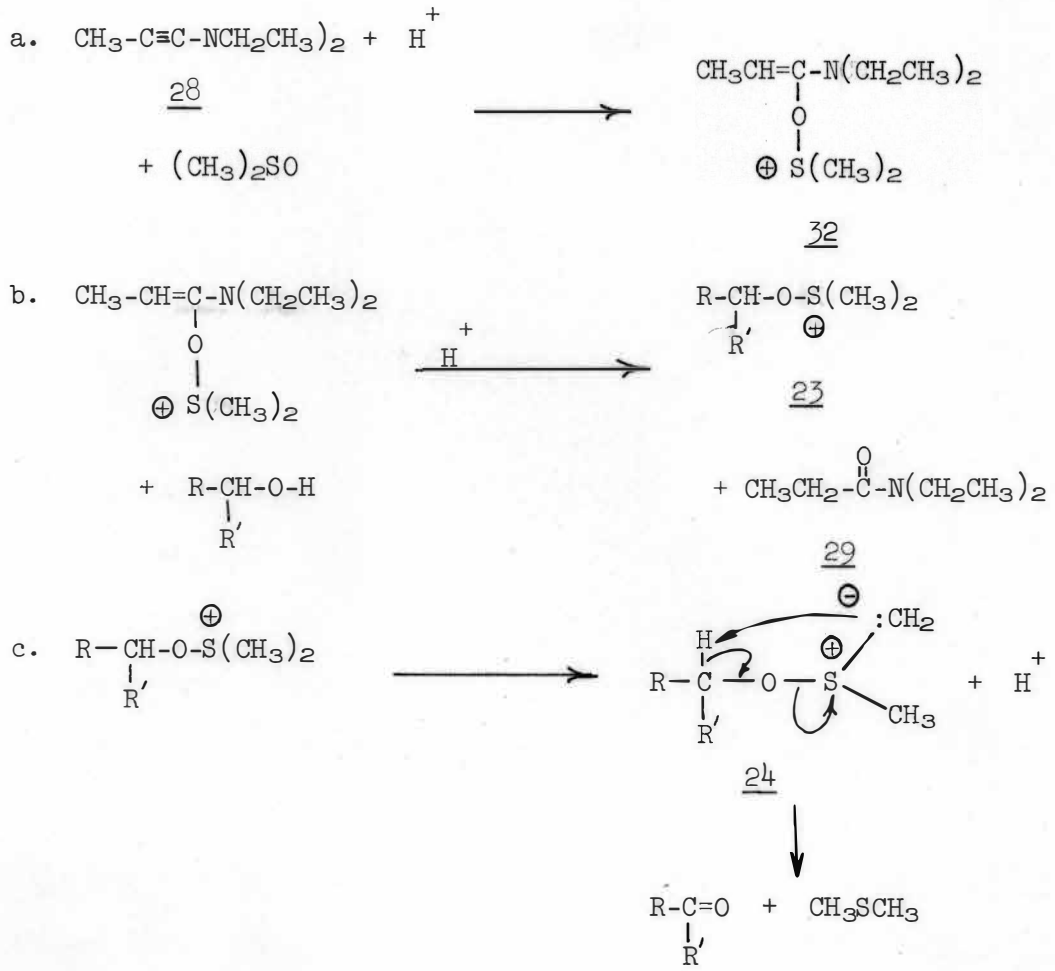
(12)

Table IV

Reactant	Product	Yield (%)
testosterone (9)	androst-4-ene-3,17-dione (10)	70
11α-hydroxy-progesterone (11)	11-keto-progesterone (12)	62
3β-hydroxy-androst-5-ene-17-one (30)	androst-5-ene-3,17-dione (14)	70
androst-5-ene-3β,17β-diol (13)	androst-5-ene-3,17-dione (14)	55

nism involves the formation of N,N-diethylamino-1-propyne—DMSO adduct (32). This is similar to the first step proposed for carbodiimide-DMSO and ketenimine-DMSO oxidations. A similar reaction was reported earlier (Scheme IV, p. 6). In this particular case, the reaction is believed to proceed via the keteniminium ion (8, $RCH{=}\overset{\oplus}{C}{-}NR'_2$). The second step (step b) involves either a stepwise or simultaneous protonation and nucleophilic attack by the alcohol molecule upon the adduct 32 with the formation of alkoxysulfonium ion 23 and N,N-diethylpropionamide (29, in guantitative yield based on the starting material 28). Such an attack is again analogous to the S_N2 displacement of hydroxysulfonium salts (hydrolysis [8,9] and alcoholysis [16]). The final step in the mechanism is the abstraction of a proton from the S-CH_3 part

Scheme VIII

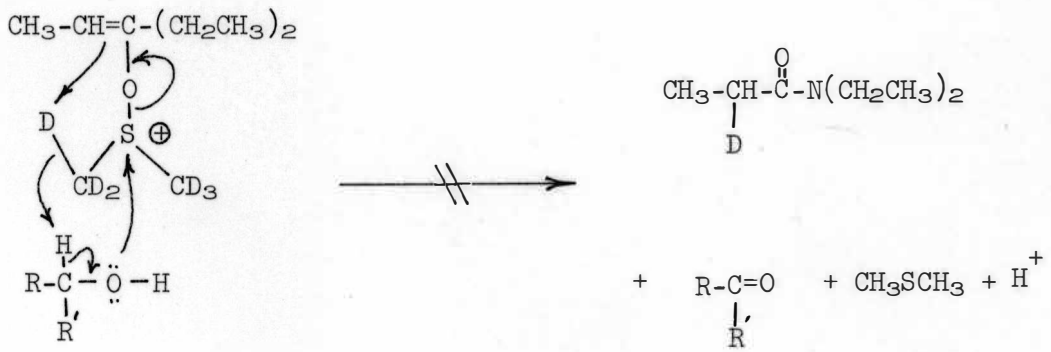


of the ion 23 and concerted collapse of the resulting sulfur ylid 24 to the carbonyl compound and dimethyl sulfide. The rationale for the last two steps is similar to that used to explain the mechanism of ketenimine-DMSO oxidation. Similarly, additional evidence for the intramolecular proton abstraction was obtained by oxidizing testosterone in the presence of DMSO-d_6, N,N-diethylamino-1-propyne, and 100% orthophosphoric acid. The nmr spectrum at 10° of the dimethyl sulfide isolated from this reaction showed a multiplet at δ 1.88 ppm which is attributed to CD_3SCD_2H. The mass spectrum of the complex obtained by

treating the above dimethyl sulfide with mercuric chloride showed an intense peak at m/e 67 which corresponds to the molecular ion $[CD_3SCD_2H]^+$ and a low intensity peak at m/e 68 attributed to the molecular ion $[CD_3SCD_3]^+$. The relative intensities of the two peaks indicated that only about 10% of the latter species was present in the mixture. Other intense peaks in the spectrum were: m/e 49, $[CD_2HS]^+$; 50, $[CD_3S]^+$; 200, $[Hg]^+$; 270, $[HgCl_2]^+$. It is believed that the CD_3SCD_3 contaminant came from the direct conversion of N,N-diethylamino-1-propyne to the corresponding amide or the decomposition of DMSO. This was further substantiated by the fact that when N,N-diethylamino-1-propyne was treated with DMSO-d_6 in the presence of 100% orthophosphoric acid, the resulting dimethyl sulfide was pure CD_3SCD_3.

An alternative mechanism similar to that suggested by Torssell for carbodiimide-DMSO oxidation (Scheme IX) was ruled out by the absence of any C-D absorption in both the infrared and the nmr spectra of N,N-diethylpropionamide (29) isolated from the above reaction.

Scheme IX



CONCLUSION

In this investigation the reagent sulfoxide-ketenimine has been used successfully to oxidize several hydroxy-steroids with yields ranging from 60-80%. N,N-Diethylamino-1-propyne and N,N-dimethylamino-phenylacetylene were also used in conjunction with DMSO and orthophosphoric acid to oxidize various hydroxy-steroids, the latter affording a better yield of the keto-steroids (50-70 as compared to 45-60%). The reaction products were characterized by infrared, ultraviolet, nmr, and mass spectroscopy.

Mechanisms for ketenimine-DMSO and ynamine-DMSO oxidations have been proposed. These mechanisms have been substantiated by deuterium labeling experiments. In addition, the stepwise mechanism proposed by Moffatt et al. for carbodiimide-DMSO oxidation has been substantiated while refuting the concerted mechanism proposed by Torssell.

Finally, it is clear from the present investigation that all the three oxidations: DMSO-DCC, DMSO-ketenimine, and DMSO-ynamine, follow identical mechanisms.

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INTRODUCTION

Purine and pyrimidine-5′-carboxylic acids have been recently isolated from the stepwise degradation of polynucleotides.[1] Therefore in order to characterize the polynucleotide degradation products, both chemical and catalytic methods have been developed to effect the oxidation of polynucleosides. [2,3] In addition, nucleoside-5′-carboxylic acids may be conjugated to amino acids to provide new synthetic antigens. [4]

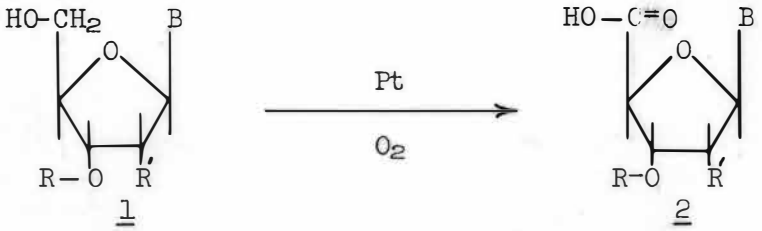
The purpose of this investigation was to develope a method for oxidizing the 5′-hydroxy group in various pyrimidine and purine nucleosides. Two general procedures involving chemical as well as catalytic oxidation have been investigated to achieve this purpose.

PART II

STUDIES ON THE OXIDATION OF THE 5'-HYDROXY GROUP OF NUCLEOSIDES

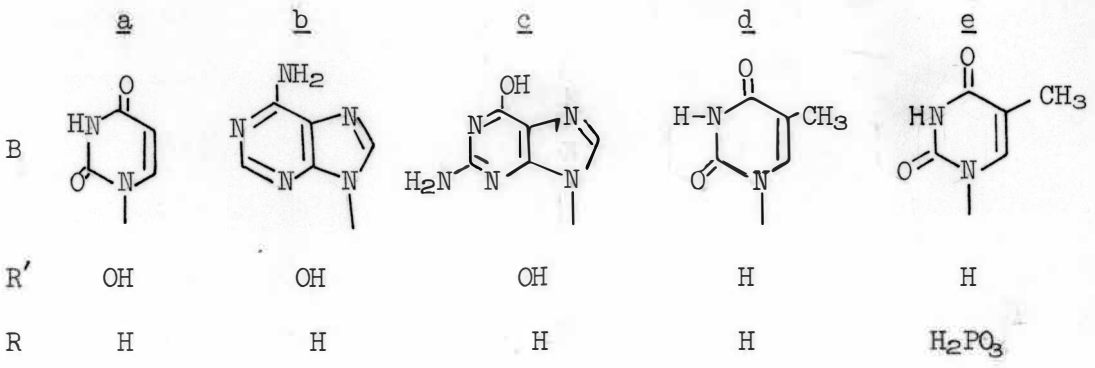
HISTORICAL

In order to characterize the products of the stepwise degradation of deoxypolynucleotides and prepare synthetic antigens, several methods have been developed to effect the oxidation of the 5′-hydroxy groups in nucleosides and nucleotides. For instance, Todd and co-workers [2] have developed a method for oxidizing the 5′-hydroxy groups in nucleosides and nucleotides. Their method consists of heating the alkaline (pH 9) solution of the nucleoside at 80° in the presence of reduced platinum oxide and oxygen. Using this method nucleosides uridine (1a), adenosine (1b), thymidine (1d), and nucleotide thymidine-3′-phosphate (1e) were oxidized to the corresponding 5′-carboxylic acids (eq 1) in yields ranging from 54-78%.



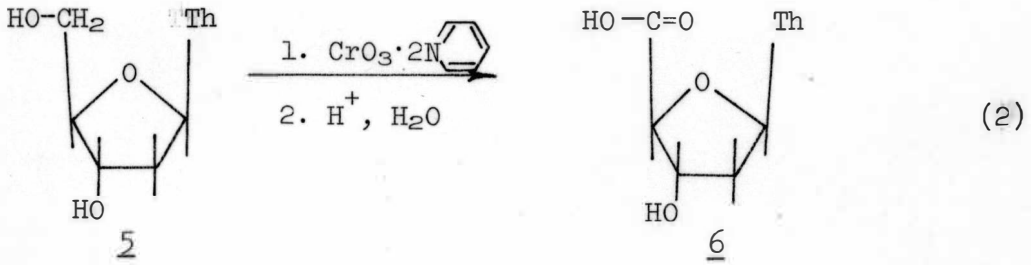
1 2 (1)

where:



Vizsolji and Tener [1] modified this procedure by conducting the oxidation of nucleosides in the presence of hydrogen peroxide instead of oxygen. Using this method, the 5′-hydroxy group in thymidine-(3′→5′)-thymidylic acid was oxidized to the corresponding carboxylic acid.

Jones and Williamson [3] achieved the oxidation of several nucleosides (e.g. thymidine, uridine, and deoxyadenosine) to the corresponding 5′-carboxylic acids using chromium trioxide-pyridine complex at room temperature (eq 2). They also tried to use manganese dioxide



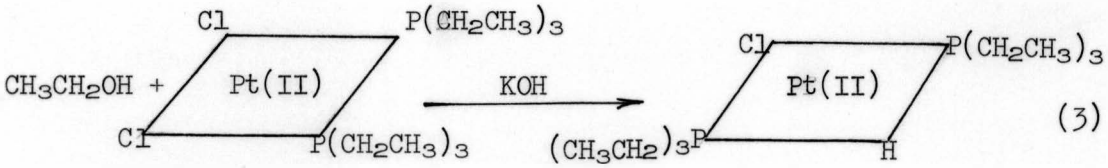
5 6 (2)

where Th=thymine

as an oxidizing agent but it was shown to be ineffective for thymidine and cause decomposition of thymidine-5′-phosphate to thymine and an unknown product. [6] Similarly, deoxyadenosine, uridine (1a), and guanosine were subjected to this oxidation procedure but the pure products could not be isolated.

Recently, the use of soluble transition metal complexes in homogeneous oxidations has been studied. For instance, Chatt and Shaw [8] have reported the oxidation of ethanol to acetaldehyde by heating the former with cis-dichlorobis(triethylphosphine)platinum(II) (7) in the presence of potassium hydroxide. The other product of the reaction

was trans-hydridochlorobis(triethylphosphine)platinum(II) (8, eq 3).



(3)

I 8

Several other oxidation methods have been developed mostly in connection with the oxidation of carbohydrates. Thus Whistler and co-workers[9] studied the oxidation of D-glucose and D-arabinose using sodium hypochlorite. They demonstrated that D-glucose is readily converted to D-gluconate ion by oxidation with alkaline hypochlorite solution. In addition, Nakagawa et al.[12] reported the successful oxidation of primary alcohols to the corresponding carboxylic acids using nickel peroxide in aqueous alkaline (pH 11) solution.

EXPERIMENTALS

Melting points were taken on a Thomas-Hoover melting point apparatus and are corrected. The elemental analyses were performed by Galbraith Laboratory Inc., Knoxville, Tennessee and Midwest Microlab Inc., Indianapolis, Indiana. A Beckman IR-8 spectrophotometer was used to record the infrared spectra. All specific rotations were determined on a standard Model D Keston Photometric Polarimeter unit attached to a Beckman DU spectrophotometer. The nmr spectra were obtained with a Varian A-60 spectrometer. Silica Gel G and cellulose powder (Darmstadt) were used for thin-layer chromatography. Spots on thin-layer chromatography plates were detected by ultraviolet light, iodine vapor or by 6 N sulfuric acid spray followed by heating at 100°.

The nucleosides used in this investigation were obtained from National Biochemical Laboratory and National Institutes of Health, Bethesda, Maryland, and were dried over phosphorous pentoxide at 100° under reduced pressure prior to use.

The stock solution used for the preparation of 2′,3′-O-isopropylidenenucleosides was prepared by adding 100g of fused zinc chloride to a liter of purified acetone (distilled over potassium permanganate, dried over anhydrous sodium sulfate, and redistilled).

Preparation of Starting Materials

General procedure for the preparation of 2',3'-O-isopropylidene derivatives of nucleosides

The desired isopropylidene derivatives were prepared according to the method of Baddilley [11] and are exemplified by the preparation of 10a. Adenosine (1a, 0.80 g, 3 mmole) was dissolved in 250 ml of the zinc chloride-acetone solution. The slightly cloudy reaction mixture was kept at room temperature for at least 20 hr, after which it was evaporated under reduced pressure to about one third of the original volume. The concentrated solution was poured into 1 l of warm (40°) barium hydroxide solution (150 g in 1 l of water). The resulting solution was cooled to room temperature and carbon dioxide gas passed through the solution until it was no longer basic. The mixture was filtered and the precipitate washed first with boiling water (350 ml) and then with warm methanol (350 ml). The filtrate and washings were evaporated at 40° to about one third of its original volume. During the evaporation, the 2',3'-O-isopropylidene derivative began to crystallize. The mixture was allowed to stand overnight and the white-crystalline material collected by filtration. The product was recrystallized from boiling methanol (95%) affording white needle-like crystals (0.54 g, 59%) of 10a, mp 219-221°, lit. [11] mp 220°.

Preparation of 9-(2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl)guanine (14)[14]

Benzoyl chloride (147 g, 1.05 mole) was added dropwise to a well-stirred solution of guanosine (60 g, 0.21 mole) in anhydrous pyridine (1.5 l). The solution was heated for 2 hr. Then the solution was

concentrated to 400 ml by evaporation under reduced pressure at 70°. The resulting mixture was poured into 2 l of water to afford a dark oil which solidified after several washings with cold water. The crude product was washed with ethanol and dried to give 14 as a light brown solid (92 g), mp 220-230°. This material was used directly in the preparation of 15.

Preparation of 2-amino-6-mercapto-9-(2′,3′,5′-tri-O-benzoyl-β-D-ribofuranosyl)purine (15)

The above material (14, 40 g) was dissolved in pyridine (1.5 l) and to the well stirred solution was added 57 g of phosphorous pentoxide. A small amount of water (4.2 ml) was added to it dropwise to prevent the formation of a tarry residue. The resulting suspension was heated under reflux for 6.5 hr (during this period several drops of water were added to ensure turbidity). The reaction mixture was kept at 0° overnight and concentrated under reduced pressure. The resulting sirup was poured into boiling water (1.5 l) to afford a sticky material which solidified on trituration with hot water and cooling. Crystallization from methanol afforded 15 as a cream-colored material (36 g, 88%), mp 215-218°. One recrystallization from the same solvent afforded yellow crystals of 15, mp 223-226.5°, lit.[14] mp 223.5-227°.

Preparation of 2-amino-6-mercapto-9-(β-D-ribofuranosyl)purine (16)

A freshly prepared solution of sodium methylate (6.5 g, 120 mmole) in 300 ml of methanol was added to a well-stirred suspension of crude 15 (37 g, 60 mmole) in 1.5 l of hot methanol. The mixture

was heated under reflux for 2 hr. During this period the pH of the solution dropped from ca 10-11 to 8.5. The residue, obtained after the removal of the solvent under reduced pressure, was dissolved in 250 ml of hot water and immediately subjected to steam distillation in order to remove methyl benzoate. The aqueous residue was filtered and acidified with glacial acetic acid to pH 5 to give a tan-colored powder which after decolorization (Norit) and crystallization from water, afforded light yellow crystals of 16 (8 g, 50%), mp 225-227° (effervescence) lit.[14] mp 224-227°. A mixture melting point with an authentic sample (mp 224-227°) showed no depression.

Preparation of 2-amino-9-(β-D-ribofuranosyl)purine (17)[14]

Thioguanosine (16) (2 g, 6.6 mmole) was dissolved in 50 ml of boiling water and treated with approximately 3 g of Raney nickel (grade W-2). On heating under reflux for approximately 2 hr, the reduction was completed as evidenced by the shift in the ultraviolet absorption maximum from 330 mμ to 294 mμ.[14] The catalyst was removed by filtration through Celite and extracted several times with boiling water. The filtrate and washings were combined, decolorized (Norit), and concentrated under reduced pressure. The resulting sirup was dissolved in hot absolute ethanol and the solvent evaporated The residual sirup was dissolved in absolute ethanol and distilled. with anhydrous benzene until a yellow amorphous powder (1.6 g, 91%) was obtained. Crystallization from absolute ethanol afforded a cream-colored product 17 (1.4 g, 79.5%). This material begins to melt slowly to an opaque glass at 109° which becomes clear

at 165°; $[\alpha]_D^{25}$ -41° (c 1.2 water).

Preparation of 2-amino-9-(2', 3'-O-isopropylidene-β-D-ribofuranosyl) purine (10g)

2-Amino-9-(β-D-ribofuranosyl)purine (1.6 g, 6 mmole), which was previously dried over phosphorous pentoxide for 4 hr at 70°, was suspended in dry acetone (150 ml). To the well-stirred solution was added 50 ml of dry 2,2-dimethoxypropane followed by the addition of several crystals of anhydrous p-toluenesulfonic acid (ca 5 mg) as a catalyst. The well-stirred suspension was refluxed for 7 hr and allowed to stand overnight at room temperature. Evaporation of the solvent afforded a crude-yellow powder which was washed with anhydrous ether (20 ml) to remove the p-toluenesulfonic acid. Recrystallization from absolute ethanol-ether (9:1) afforded tan-colored crystals of 10g (0.96 g, 50%), mp 170-175°.

Preparation of 2',3'-O-isopropylidenethioguanosine (10e)

p-Toluenesulfonic acid (ca 5 mg) was added with stirring to a suspension of thioguanosine (3.2 g, 10 mmole) in a mixture of dry acetone (210 ml) and 2,2-dimethoxypropane (50 ml). After heating under reflux for 20 min, a homogeneous solution was obtained which was further refluxed for an additional 2 hr and allowed to stand overnight. The solvent was evaporated under reduced pressure and the crystalline residue was washed with ether to remove the p-toluenesulfonic acid. After drying, a yellow solid was obtained (softens at 136-145° and effervesces to a clear liquid at 160°). Crystallization from ethanol afforded 2.2 g (61%) of yellow crystals of

10e, mp > 265°. Nmr spectrum: δ 1.35 (3, s, C-$\underline{CH_3}$), 1.55 (3, s, C-$\underline{CH_3}$), 3.65 (2, d, $\underline{H_5}$), 4.23 (1, m, $\underline{H_4}$), 5.2 (3, m, $\underline{H_2}$, $\underline{H_3}$, $\underline{HS}$), 6.06 (1, d, $\underline{H_1}$), 6.93 (2, s, $\underline{NH_2}$), and 8.24 (1, s, $\underline{H_8}$).

Preparation of 2′,3′-O-isopropylidene-S-methylthioguanosine (10f)

2′,3′-O-Isopropylidenethioguanosine (10e, 0.8 g, 2.5 mmole) was dissolved in 3 ml of 0.4 N sodium hydroxide and to the resulting solution was added dropwise 0.14 ml of methyl iodide with stirring. A yellow semi-solid separated out of the solution. The solvent was decanted and the yellow residue washed with water and dried over calcium chloride to give 10f (0.6 g, 83%). Attempted crystallization from absolute ethanol afforded 0.4 g (55.5%) of a tan-colored powder. Nmr spectrum (δ): 1.35 (3, s, $\underline{CH_3}$), 1.55 (3, s, $\underline{CH_3}$), 2.6 (3, s, S-$\underline{CH_3}$), 3.56 (2, d, $\underline{H_5}$), 4.24 (1, m, $\underline{H_4}$), 5.2 (2, m, $\underline{H_2}$, $\underline{H_3}$), 6.08 (1, d, $\underline{H_1}$), 6.5 (2, s, $\underline{NH_2}$) and 8.5 (1, s, $\underline{H_8}$).

Preparation of 2′,3′-O-isopropylideneguanosine (10b)

2′,3′-O-isopropylideneguanosine was prepared according to the previously described general procedure. Dry guanosine (7.4 g, 26 mmole) was treated with 22.2 g of fused zinc chloride dissolved in 200 ml of anhydrous acetone. However, only a small amount of the expected product was obtained after the usual work-up. Therefore, the resulting precipitate was extracted with an aqueous solution of potassium hydroxide (pH 11). Evaporation and neutrallization of the extract afforded 10b as a white crystalline product (6 g, 70%), mp 270-276°. Recrystallization from ethanol gave 5.4 g (63%) of pure 10b, mp 298-300°, lit.[11] mp 300°.

Oxidation of 2′,3′-O-Isopropylidenenucleosides Using Alkaline Permanganate

The general procedure can be illustrated by the oxidation of 2′,3′-O-isopropylideneadenosine. 2′,3′-O-Isopropylideneadenosine (10a, 2.34 g, 10 mmole) was dissolved in warm water (800 ml). The solution was cooled to room temperature and potassium hydroxide (1.17 g, 30 mmole) was added to it with stirring. Then a solution of potassium permanganate (4.74 g, 30 mmole) in water (150 ml) was added to it dropwise over a period of 2 hr. The mixture was stirred for 3 days at room temperature. Excess permanganate was destroyed with hydrogen peroxide. The precipitated manganese dioxide was removed by filtration through Celite. The colorless filtrate was concentrated under reduced pressure at 40° to approximately 100 ml. Acidification of the resulting solution at 0° to pH 5 afforded a white powder which after crystallization from water gave colorless crystals (2.1 g, 80.2%) of 11a, mp 268-269°. The analytical sample was obtained by one recrystallization from water, mp 277-279°; $[\alpha]_D^{25}$ -109 (c 1.0, 1 N NaOH); λ_{max}^{KBr} (cm^{-1}) 3300 (OH, NH), 1625 (C=N), 1720 (C=O), and 1375 ($C(CH_3)_2$). Anal. calcd. for $C_{13}H_{15}N_5O_5$: C, 48.57; H, 4.67; N, 21.86. Found: C, 48.74; H, 4.67; N, 21.70.

Hydrolysis of 2′,3′-O-isopropylideneadenosine-5′-carboxylic acid (11a)

Hydrochloric acid (20 ml, 1 N) was added to a suspension of 0.5 g of 11g in 20 ml of water. The solution was warmed to 40° and the reaction was followed by thin-layer chromatography using 2-propanol-ammonium hydroxide (7:3) as the developing solvent system. The

hydrolysis was over after 10 min. The solution was then cooled to 5° and the pH was adjusted to 4.5 by the addition of 30% sodium hydroxide. This yielded 0.3 g of crude 14, mp 300-305°. Reprecipitation by first dissolving the crude solid in aqueous sodium hydroxide and then adding dilute sulfuric acid, gave 0.2 g of pure adenosine-5'-carboxylic acid (14), mp $>$ 320°; $[\alpha]_D^{25}$ -11° (c 2, 0.1 N NaOH); lit.[2] $[\alpha]_D^{25}$ -14° (c 2, 0.1 N NaOH), mp $>$ 320°.

Oxidation of 2-amino-2',3'-O-isopropylidene-9-(β-D-ribofuranosyl)-purine (10g)

2-Amino-2',3'-O-isopropylidene-9-(β-D-ribofuranosyl)purine (10g, 0.76 g, 2.5 mmole) was dissolved in 600 ml of water and treated with potassium permanganate (1.65 g) following the general procedure described earlier. After the usual work-up procedure, a white amorphous powder (0.65 g) was obtained which on crystallization from absolute ethanol afforded colorless crystals (0.3 g, 30%) of 11g, mp 227-228°. The analytical sample was obtained after one recrystallization from absolute ethanol; ν_{max}^{KBr} (cm^{-1}) 3320 (OH,NH), 1680 (C=O), 1625 (C=N), and 1375 ($C(CH_3)_2$); $\lambda_{max}^{H_2O}$ 260 mμ (ε 2,512). Anal. calcd. for $C_{13}H_{15}N_5O_5$: C,48.57; H, 4.67; N, 21.86. Found: C, 48.28; H, 4.68; N, 21.52.

The isopropylidene function was removed by hydrolysis using 1 N HCl (1 ml) at 45° for 10 min. The resulting solution was spotted on a Whatman number 1 paper and chromatographed using water saturated with butanol as the developing solvent system. The spots were detected by spraying with 5% periodate solution (aqueous) followed by ammoniacal silver nitrate spray (5% solution in 95%

ethanol). A brown spot, R_f=0.7, was obtained (R_f=0.83 for the starting material 17).

Attempted oxidation of 2',3'-O-isopropylideneguanosine (10b)

2',3'-O-Isopropylideneguanosine (10b, 2.3 g, 7 mmole), and potassium permanganate (4.4 g, 20 mmole) were reacted according to the general procedure. Acidification of the resulting concentrate failed to give the expected acid. Therefore, the solution was treated with calcium chloride to give a white precipitate (1.1 g), which was stirred with Dowex-50 (H^+) for 1 hr and filtered. Concentration of the solvent to ca 25 ml, afforded some colorless crystals (200 mg), mp 187-188°. These crystals were subsequently identified to be oxalic acid on the basis of undepressed melting point of the mixture of this material with an authentic sample of anhydrous oxalic acid.

Attempted oxidation of 2',3'-O-isopropylideneinosine (10c)

2',3'-O-Isopropylideneinosine (10c, 2.2 g, 7 mmole) was treated with potassium permanganate (4.74 g, 30 mmole) at pH 12 under conditions similar to those described earlier. As in the case of the guanosine derivative, no precipitate was obtained after acidification. Treatment of the resulting solution with calcium chloride gave the usual white precipitate (450 mg), mp 100-104°. Sublimation of this material gave colorless crystals (250 mg), mp 187-188°. Infrared and nmr spectra of these crystals were similar to those of anhydrous oxalic acid.

Attempted oxidation of 2',3'-O-isopropylideneuridine (10d)

The compound 10d (0.4 g, 1.5 mmole) was oxidized using alkaline potassium permanganate (1.8 g, pH 12) using the same general procedure. The results were similar to those obtained from the oxidation of 10b and 10c. In this case, 50 mg of oxalic acid was isolated from the reaction mixture.

Attempted oxidation of 2',3'-O-isoproylidenethioguanosine (10e)

Oxidation of 2',3'-O-isopropylidenethioguanosine (10e, 2.1 g, 6.8 mmole) was attempted using the usual procedure. After concentration of the resulting solution, acidification of the concentrated solution provided 0.4 g of a tan-colored solid, mp 99-140°. Infrared spectrum of this material showed a rather weak absorption band at 1690 cm^{-1} due to the presence of a carbonyl group. However, after two recrystallizations from ethanol, the material responsible for the above infrared absorption was removed leaving 300 mg of pure 2',3'-O-isopropylidenethioguanosine. Infrared spectrum and melting point of this material were identical with those of an authentic sample. Evaporation of the solvent from the above filtrate gave a yellow solid (100 mg), mp 140-170° whose infrared spectrum showed a strong absorption at 1690 cm^{-1}. However, attempted isolation of the pure product was unsuccessful.

Attempted oxidation of 2',3'-O-isopropylidene-S-methylthioguanosine (10f)

The oxidation of 2',3'-O-isopropylidene-S-methylthioguanosine (10f, 0.3 g, 0.85 mmole) was attempted by following the general

oxidation procedure. Work-up yielded 0.2 g (66%) of the unreacted starting material and no oxidation product.

Other Oxidizing Agents Used

Attempted oxidation of 2',3'-O-isopropylideneadenosine using cis-dichlorobis(triphenylphosphine)platinum (II) in the presence of oxygen

2',3'-O-Isopropylideneadenosine (10a, 0.51 g, 1.5 mmole) was added to 1.3 g (1.5 mmole) of cis-dichlorobis(triphenylphosphine)-platinum (II) (7) in 600 ml of dry acetone. The solution was refluxed for 24 hr while air was bubbled through the solution. The suspension was concentrated and the residue extracted with warm chloroform (150 ml). The tan-colored residue (0.3 g) was crystallized from acetone to give white crystals (0.2 g), mp 216-219°. Recrystallization from acetone afforded colorless crystals of the starting material (10a) (0.12 g), mp 219-220°.

Attempted oxidation of 2',3'-O-isopropylideneadenosine using chlorotris(triphenylphosphine)rhodium(I) and oxygen

2',3'-O-Isopropylideneadenosine (10a, 0.25 g, 0.75 mmole) was dissolved in 100 ml of acetone and a solution of chlorotris(triphenylphosphine)rhodium(I) (12, 0.65 g, 0.75 mmole) in 100 ml of acetone was added to it. Few drops of triethylamine were also added and the solution was refluxed for 14 hr while bubbling a continuous stream of air through it. After that, the solvent was evaporated and the residue extracted with base (pH 11). Concentration and acidification (dil. HCl at 0°) of the resulting solution afforded a white crystalline material (0.16 g), mp 218-220° whose infrared spectrum was

superimposable with that of the starting material.

Attempted oxidation of 2',3'-O-isopropylideneguanosine using nickel peroxide

Sodium hydroxide (0.5 g) was added to a suspension of 2',3'-O-isopropylideneguanosine (1.12 g, 3.4 mmole) in water (250 ml). The solution became homogeneous at this point. To this solution was added nickel peroxide (2.08 g, 13 mmole) and the mixture was allowed to react overnight at 40°. The resulting greenish-black residue was filtered and washed with water (100 ml). The filtrate and washings were concentrated at 40° under reduced pressure. Acidification of the residue to pH 5 at 0° afforded a cream-colored precipitate (0.6 g). Crystallization from absolute ethanol gave a white crystalline material (0.4 g), mp 328-300°. A mixture melting point of this material with an authentic sample of 10b was undepressed. Infrared spectrum of the two samples were superimposable.

Attempted oxidation of 2',3'-O-isopropylideneadenosine (10a) using sodium hypochlorite

2',3'-O-Isopropylideneadenosine (10a, 0.6 g, 2 mmole) was dissolved in 14 ml of sodium hypochlorite (6%) at 5°. The resulting red solution was extracted with chloroform (3 x 250 ml) after 15 min. Evaporation of the solvent afforded a red-orange oil (0.3 g) which did not solidify even after dissolving in absolute ethanol and subsequent azeotrope distillation with benzene to remove any water. Infrared spectrum of this sirup showed a very strong absorption at 1725 cm^{-1}. Thin-layer chromatography on Silica Gel G using (a) water saturated with butanol, (b) isopropanol-ammonium hydroxide

(7:3), and (c) butanol-water-pyridine (3:6:1) systems showed three spots.

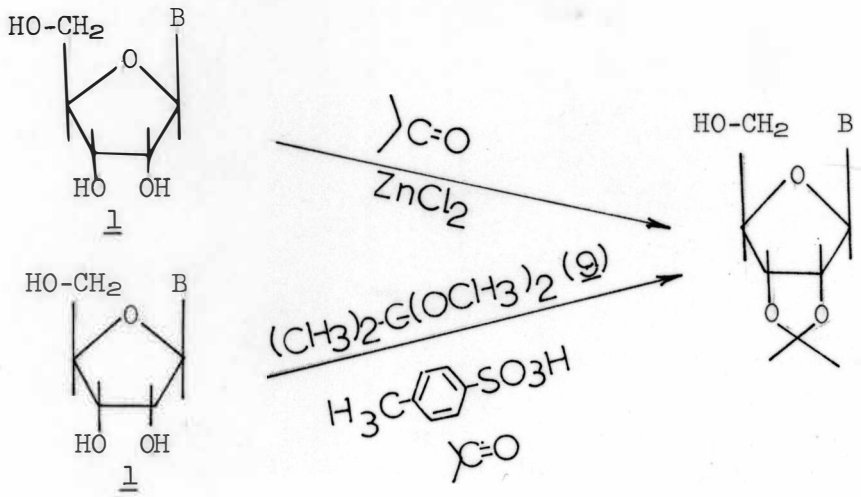
Attempted oxidation of 2',3'-O-isopropylideneadenosine using sodium hypobromite as oxidant

2',3'-O-Isopropylideneadenosine (10a, 0.51 g, 1.8 mmole) was added to a solution prepared by mixing 50 ml of 2% sodium hypochlorite, 1.6 g of potassium bromide, 2.8 g of potassium hydrogen phosphate, and 3 g of potassium phosphate. The reaction mixture was allowed to react for 10 min after which it was extracted with chloroform (3 x 150 ml). The chloroform solution was washed twice with water (50 ml) and evaporated. A sirupy material (0.3 g) was obtained whose infrared spectrum showed a strong absorption at 1730 cm^{-1} but which failed to crystallize when dissolved in absolute ethanol and distilled with benzene. Isolation of the pure material was unsuccessful due to the presence of several other unidentifiable products.

DISCUSSION

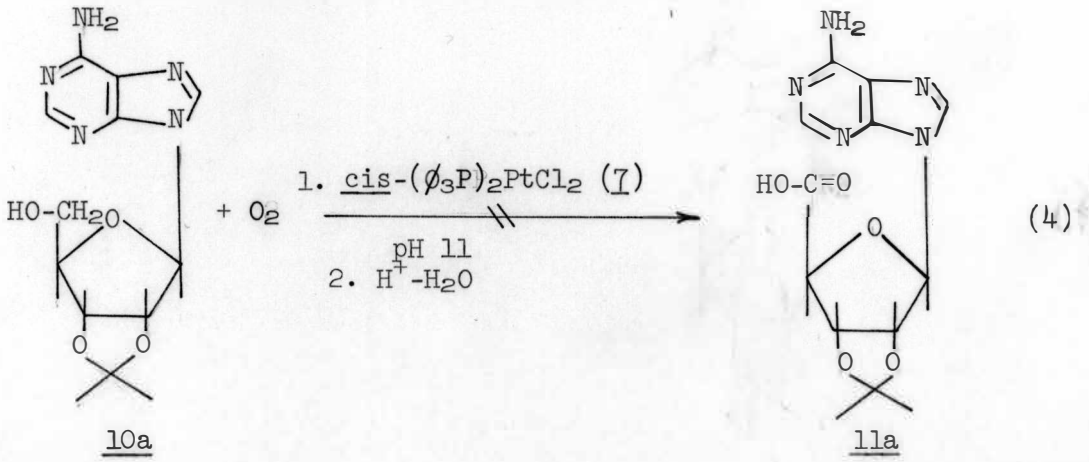
Apparently, all the methods available to date for the oxidation of nucleosides work better for pyrimidine rather than purine-nucleosides.[1-3] In this investigation, several methods (catalytic as well as chemical) were studied with the hope of developing a procedure which could be suitable for the oxidation of both types of nucleosides, or at least for purine-nucleosides. The 2',3'-hydroxy groups in the starting nucleosides were protected by converting them to the corresponding 2',3'-O-isopropylidene derivatives. Two procedures were used to prepare these isopropylidene derivatives. The first procedure was similar to that reported by Baddiley.[11] The second procedure involved treating the nucleosides with 2,2-dimethoxypropane (9) in acetone in the presence of catalytic amount of p-toluenesulfonic acid (Scheme I).

Scheme I



where B= purine or pyrimidine base

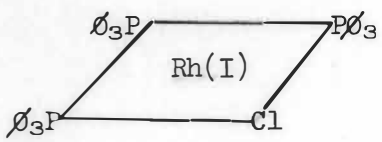
Oxidation of 2',3'-O-isopropylideneadenosine (10a) was attempted by the method of Chatt and Shaw.[8] Using this method, an alkaline solution (pH 11) of the nucleoside 10a was heated at 80° in the presence of catalytic amount of cis-dichlorobis(triphenylphosphine) platinum(II) (7) while air was being bubbled through the solution for 14 hr (eq 4). Unfortunately, no oxidized product was obtained



(4)

10a 11a

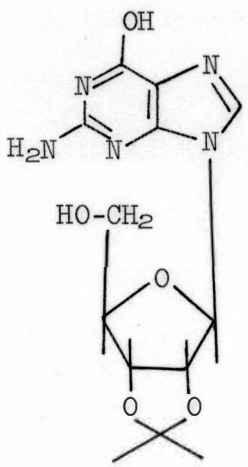
even after changing the solvent from water to acetone. A similar method which made use of chlorotris(triphenylphosphine)rhodium(I) (12) instead of 7 was tried but as before (eq 4), no oxidation was observed when 2',3'-O-isopropylideneadenosine was subjected to this procedure.



12

In 1962, Nakagawa and co-workers[12] had reported the use of alkaline nickel peroxide for the oxidation of simple primary alcohols

to the corresponding carboxylic acid. Using this method, an aqueous alkaline solution (pH 11.5) of 2′, 3′-O-isopropylideneguanosine (10b) was treated with a four-fold excess of freshly prepared nickel peroxide and the suspension was heated at 40° for 12 hr. Only the unreacted starting material (10b) was isolated on work-up of the reaction mixture.



10b

$$\xrightarrow[\text{pH 11.5}\ 40^\circ]{NiO_2} \text{no reaction} \qquad (5)$$

Sodium hypochlorite has been reported to oxidize the hydroxyl group in several carbohydrates.[9] Therefore, we investigated the possible use of this reagent as well as sodium hypobromite in the oxidation of 2′,3′-O-isopropylidene protected nucleosides. Thus 2′,3′-O-isopropylideneadenosine (10a) was treated with 6% sodium hypochlorite at 5° for 30 min. Extraction with chloroform and evaporation of the solvent afforded an orange-colored oil, which failed to crystallize out. Infrared spectrum of this material showed a strong absorption band at 1725 cm^{-1} characteristic of a carbonyl group. However, all attempts to isolate the pure product from the

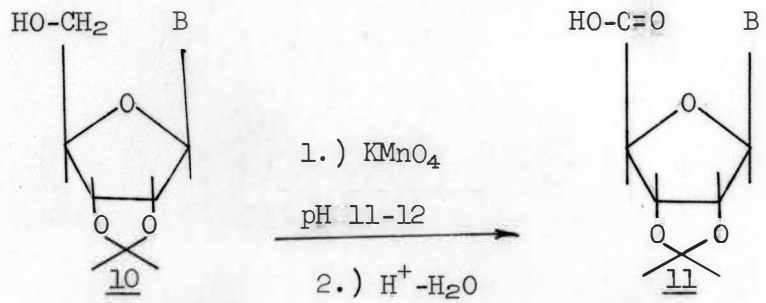
rest of the reaction mixture were unsuccessful. The use of sodium hypobromite for the oxidation of 10a afforded identical results.

In 1967, Hayatsu and Ukita [13] reported the use of potassium permanganate ($KMnO_4$) in the chemical modification of nucleic acids. However, the products of these reactions were not characterized. Since $KMnO_4$ is rather inexpensive and readily available, it should be an ideal oxidizing agent if found useful in oxidizing nucleosides. With this in mind, several nucleosides (pyrimidine and purine) were oxidized using alkaline permanganate. This method can not, of course, be applied to nucleosides containing cis-glycols because of the possibility of ring cleavage by alkaline permanganate. Therefore, it is necessary to protect the 2',3'-hydroxy groups in the nucleosides, for instance, by an isopropylidene or a benzylidene function. This oxidation procedure can be illustrated by the oxidation of 2',3'-O-isopropylideneadenosine (10a) and 2-amino-2',3'-O-isopropylidene-9-(β-D-ribofuranosyl)purine (10g).

The oxidation was conducted by adding dropwise over a period of 2.5 hr an aqueous solution of potassium permanganate to a solution of the appropriate nucleoside and potassium hydroxide (pH 11-12) in a large amount of water(ca. 1 l). The reaction mixture was stirred at room temperature and followed by thin-layer chromatography in (a) water saturated with n-butanol or (b) isopropanol-ammonium hydroxide (7:3), as the developing solvent systems. When the oxidation was over (1-2 days) the excess permanganate was destroyed by the addition of hydrogen peroxide (30%). The precipitated manganese dioxide was removed by filtration through Celite and the filtrate concentrated

under diminished pressure to about 100 ml. Acidification (pH 4.5) of the residue with dilute hydrochloric acid at 0° afforded the corresponding nucleoside-5'-carboxylic acid as an amorphous powder in 80% yield.

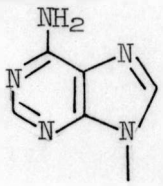
Thus, the oxidation of 2',3'-O-isopropylideneadenosine (10a) afforded 2',3'-O-isopropylideneadenosine-5'-carboxylic acid (11a, eq 6)



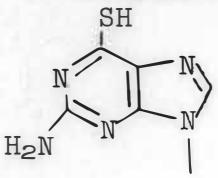
10 11 (6)

where B equals:

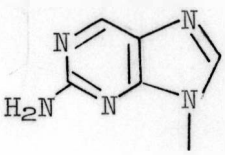
a. adenine



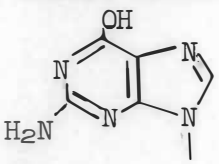
e. thioguanine



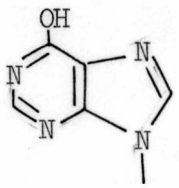
g. 2-aminopurine



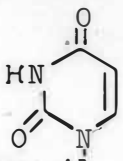
and for others tried:



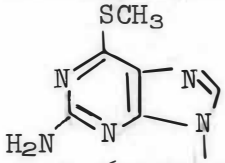
b. guanine



c. hypoxanthine

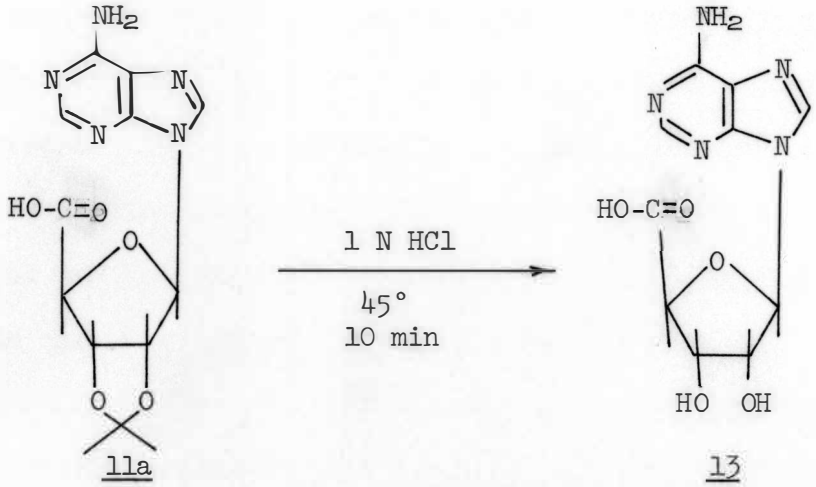


d. uracil



f. 6-methylthioguanine

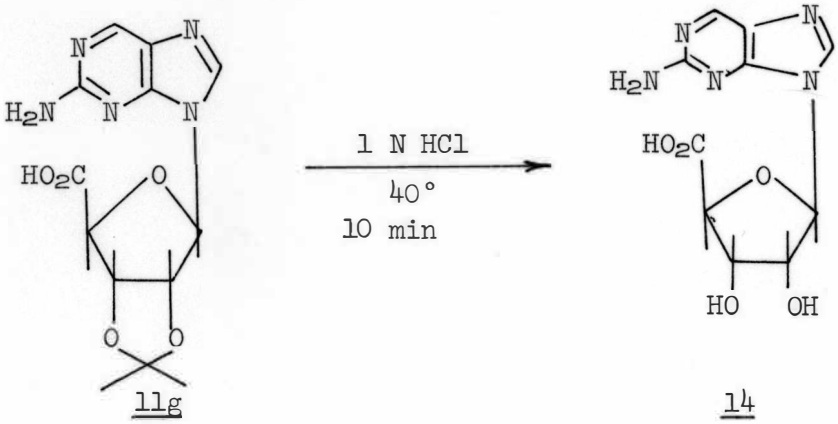
Hydrolysis of the isopropylidene function in 11a by aqueous hydrochloric acid afforded adenosine-5′-carboxylic acid (13, eq 7) whose phy-



11a 13 (7)

sical constants were in agreement with the literature value.[2]

Similarly, the oxidation of 2-amino-2′,3′-O-isopropylidene-9-(β-D-ribofuranosyl)purine (10g), prepared by Fox and co-workers,[14] furnished the corresponding 5′-carboxylic acid (11g). The isopropylidene function in 10g was hydrolyzed by aqueous hydrochloric acid to give compound 14. Paper chromatography of 14 (eq 8) relative to 2-amino-9-(β-D-ribofuranosyl)purine (17) gave a slower moving brown spot when sprayed with sodium metaperiodate solution followed by an ammo-



11g 14 (8)

niacal silver nitrate spray. This demonstrated that during the oxidation of 10g, the sugar (ribose) portion remained intact.[16]

However, when 2',3'-O-isopropylideneguanosine (10b), 2',3'-O-isopropylideneinosine (10c), and 2',3'-O-isopropylideneuridine (10d) were subjected to alkaline permanganate oxidation, the only product which could be isolated from these reactions was oxalic acid. The latter compound was isolated from the acidic solution by precipitation with calcium chloride. The nucleoside 10d was found to react with permanganate faster than the nucleosides 10c or 10b. Oxidation of 2',3'-O-isopropylidenethioguanosine (10e) with alkaline permanganate under similar conditions afforded a small amount of of some impure material whose infrared spectrum had a strong absorption at 1690 cm^{-1} indicating the presence of a carbonyl group. However, attempted isolation of the pure product was unsuccessful.

CONCLUSION

In this investigation alkaline potassium permanganate was found to be useful in the oxidation of 2′,3′-O-isopropylideneadenosine (10a) and 2-amino-2′,3′-O-isopropylidene-9-(β-D-ribofuranosyl)purine (10g).[15] The isopropylidene function in these two carboxylic acids were hydrolyzed to afford adenosine-5′-carboxylic acid and 2-amino-9-(β-D-ribofuranosyl)purine-5′-carboxylic acid. Oxidation of 2′,3′-O-isopropylideneguanosine (10e) afforded only a small amount of a carbonyl-group-containing material which could not be purified or characterized. In addition, the oxidation of 2′,3′-O-isopropylidene nucleosides 10b to 10d led to the formation of oxalic acid and other unidentifiable materials. An attempt to oxidize 10f using this method was unsuccessful due probably to the extreme insolubility of this compound in water.

The attempted oxidation of 2′,3′-O-isopropylideneadenosine using cis-dichlorobis(triphenylphosphine)platinum(II) (7) and chlorotris-(triphenylphosphine)rhodium(I) (12) proved fruitless. In both cases, unchanged starting materials were isolated.

Similarly, the oxidation of 2′,3′-O-isopropylideneguanosine using alkaline nickel peroxide was unsuccessful as only the starting material was isolated from this reaction.

Finally, when alkaline (pH 11) sodium and potassium hypochlorite solutions were used to oxidize 10a, an orange-sirupy product was obtained. The infrared spectrum of this material showed an absorption

at 1725 cm^{-1} (C=O) but all attempts to isolate the pure product from the rest of the impurities were unsuccessful.

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VITA

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