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OXIDATION STUDIES IN SOME STEROIDAL BASES (SOLASODINE AND 3 β-O-ACETYL SOLASODINE)

Part I. Reaction With Potassium Permanganate

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Oxidation of solasodine with potassium permanganate/acetic acid yielded I and II while the same reaction when carried out with 3 β-O-acetyl solasodine gave V. On the other hand treatment of solasodine with potassium permanganate in pyridine led to the formation of VII which on acetylation and subsequent hydrolysis formed the diosphenol derivative IX.

INTRODUCTION

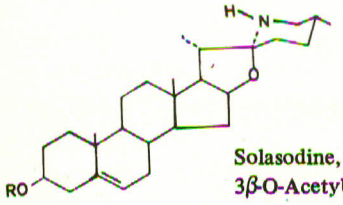
As a part of current pharmaco-chemical investigations in conessine and other steroidal alkaloids, oxidative studies have been carried out on solasodine and 3β-O-acetyl solasodine. Solasodine, which is the common hydrolysed product of the Solanum glycobases, was isolated from the ripe and unripe berries of *Solanum xanthocarpum* Schrad. and Wendle. following the procedure reported earlier[1]. As a result of the present work various new derivatives of the base have been obtained and their structures established through chemical and spectral data.

On oxidation of solasodine with potassium permanganate in acetic acid two products were obtained, the structures of which have been established as (25R) - 22α N-spirosola-4, 6-dien-3-one (I) and (25R) -6α-hydroxy-22α N-spirosol-4-en-3-one (II). I showed M^+ at *m/e* 409.30026 corresponding to the molecular formula $C_{27}H_{39}NO_2$. Its IR spectrum showed a conjugated carbonyl group at 1660 cm^{-1} and C=C stretching at 1610 and 1630 cm^{-1}. The UV specturm of this compound was very informative showing λ_{max} at 283 nm (calculated λ_{max} 280 nm for $\Delta^{4,6}$ -3-one) [2]. Its IR specturm showed absence of any hydroxyl fucntion, this was supported through its acetylation which gave N-acetylated product III with M^+ at m/e 451 and absence of fragments at *m/e* 138 and *m/e* 114 resulting from the cleavage of ring E[3]. Furthermore, the IR spectrum of III showed carbonyl absorption at 1640 cm^{-1} for amide group while UV spectrum showed same maxima as of the original compound. The ^{1}H-NMR spectrum of I (see Table) showed broad multiplet for H-4 and H-6 olefinic protons extending from δ 5.4-5.8 and H-7 olefinic proton at δ 6.2 apart from the usual signals observed in solasodine. II showed M^+ at *m/e* 427.31045 confirming the molecular formula $C_{27}H_{41}NO_3$ while the IR spectrum was indicative of a conjugated carbonyl at 1665 and 1610 cm^{-1}, which was confirmed through the UV λ_{max} at 240 nm (calculated λ_{max} 244 nm for Δ^4-3one)[2]. On its acetylation with excess of acetic anhydride in pyridine the diacetyl derivative IV was obtained showing M^+ at *m/e* 511 and absence of peaks at *m/e* 114 and *m/e* 138. Its IR spectrum showed carbonyl peaks at 1645 and 1730 cm^{-1} for the amide and the acetate functions respectively.

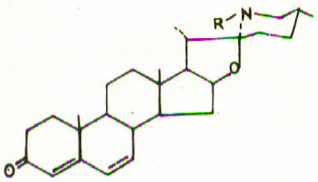
It is interesting to note that the same reaction when carried out with 3β-O-acetyl solasodine, under more or less similar reaction conditions as in the case of solasodine, it resulted in allylic oxidation at C-7 forming (25R)-7-oxo-5α-22α N-spirosolan-3β,5α,6α-triol-3-acetate (V). It showed M^+ at *m/e* 503.3237 corresponding to the molecular formula $C_{29}H_{45}NO_6$; its UV spectrum showed absence of any conjugated chromophore, while IR spectrum exhibited two carbonyl absorptions at 1715 and at 1730 cm^{-1} for 7-keto and acetyl functions. Acetylation of V with acetic anhydride and pyridine at room temperature afforded VI. Its mass spectrum showed M^+ at 587.3441 ($C_{33}H_{49}NO_8$) and absence of fragments at *m/e* 114 and *m/e* 138. Its IR spectrum revealed carbonyl peaks of acetate and keto groups at

1720, 1735, 1710 cm^{-1}, of amide at 1645 cm^{-1} and -OH stretching at 3400 cm^{-1}.

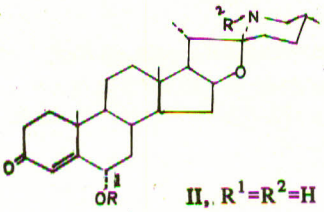
Keeping in view the above observations, reaction of solasodine was performed in presence of pyridine instead of acetic acid to find out if it undergoes allylic oxidation in this medium as noted by Nace and Rieger in case of Δ^5-steroids[4]. Analogous to their observations (25R)-3β, 5α,6 α-trihydroxy-5α, 22αN-spirosolan-7-one (VII) was obtained from solasodine in this reaction. Its mass spectrum showed M^+ at *m/e* 461.3131 corresponding to the molecular formula $C_{27}H_{43}NO_5$ while the IR spectrum was indicative of hydroxyl as well as ketonic stretchings at ~3400 cm^{-1} and 1715 cm^{-1} respectively. Furthermore, the ^{1}H-NMR is also in agreement with this structure. The structure was confirmed chemically through the formation of its diacetate VIII, which on hydrolysis with methanolic potassium hydroxide yielded (25R)-3β, 6-dihydroxy-spirosol-5-en-7-one (IX). IX gave positive ferric chloride test and its UV spectrum showed λ_{max} at 276 nm, showing the diosphenol type structure (λ_{max} calculated for diosphenol= 279 nm)[2,4]. VII on prolonged treatment with excess of acetic anhydride in pyridine at room temperature afforded (25R)-22,26-acetylepimino-7-oxo-cholest-22-en-3β, 5α, 6α, 16β-tetraol-3,6,16-triacetate (X). Its UV spectrum showed maxima at 235 characteristic of unsaturated



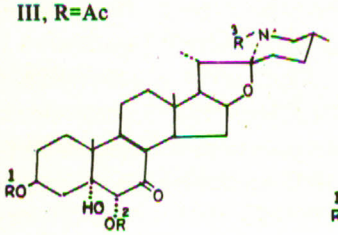
Solasodine, R=H
3β-O-Acetyl solasodine, R=Ac



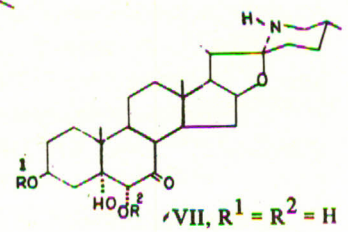
I, R=H
III, R=Ac



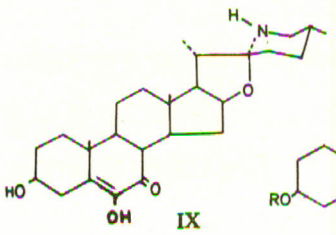
II, $R^1=R^2=H$
IV, $R^1=R^2=Ac$



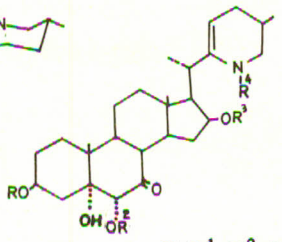
V, R^1 = Ac, R^2 = R^3 =H
VI, R^1 = R^2 = R^3 = Ac



VII, R^1 = R^2 = H
VIII, R^1 =R^2 = Ac



IX



X=R^1=R^2=R^3=R^4=Ac

Table. ^{1}H Spectral data

Proton	Solasodine	I	II	V	VII	X
19	1.02,s, 3H	1.2 s, 3H	1.2 ,s, 3H	1.25,s, 3H	1.27,s, 3H	1.25,s, 3H
18	0.82,s, 3H	0.86,s,3H	0.83,s, 3H	0.83,s, 3H	0.82,s, 3H	0.908,s,3H
21	0.94,d, 3H	0.96,d,3H	0.94,d, 3H	0.95,d, 3H	0.94,d, 3H	1.09,d, 3H
27	0.85,d, 3H	0.85,d,3H	0.85,d, 3H	0.87,d, 3H	0.87,d, 3H	0.89,d, 3H
3	3.5, m,1H	–	–	4.98,m, 1H	4.05,m,1H	4.8-5.15[b]
6	5.34,m,1H	5.4-5.8[a]	4.1, m,1H	5.1 s, 1H	5.2 , s, 1H	5.6, s, 1H
16	4.28,m,1H	4.3, m, 1H	4.25,m,1H	4.28,m,1H	4.26,m,1H	4.8-5.15[b]
26	2.62,m,2H	2.60,m,2H	2.60,m,2H	2.62,m,2H	2.63,m,2H	2.8-3.5,m,2H
4	–	5.4-5.8[a]	5.9, d, 1H	–	–	–
7	–	6.2 ,m,1H	–	–	–	–
23	–	–	–	–	–	5.3 ,m,1H
Acetyl				2.02,s, 3H		2.03,9H and 2.08, 3H (3xOAc and N-Ac)

a) H-6 and H-4 appeared as 2-protons broad multiplet; (b) H-3 and H-16 appeared as 2-protons multiplet; All values are in δ (ppm) relative to TMS=0.

acetyl-amino function[5]. Its IR exhibited a broad peak between 1710-1735 for acetates and ketonic functions, a peak of amide carbonyl at 1660 and C=C at 1640 cm^{-1}. The mass spectrum showed M^+ at 629.3552 ($C_{35}H_{51}NO_9$) and fragments at *m/e* 587, 544, 166 (B.P), 83, while the ^{1}H-NMR spectrum showed vinylic proton at δ 5.3 and four acetyl groups at δ 2.03 (9H) and δ 2.08 (3H).

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. IR and UV spectra were measured on IRA-1 Diffraction Grating Infrared Spectrometer and Shimadzu VU - Vis Recording Spectrophotometer UV-240 respectively. All UV spectra were taken in methanol solution. Proton NMR spectra were recorded in $CDCl_3$ on Bruker WP 100sy with TMS as internal reference. Mass spectra were recorded on MAT 112 and Varian MAT 312 spectrometers. The purity of the samples was checked on TLC (silica gel).

Potassium Permanganate-Acetic Acid Oxidation of Solasodine – (25R)-22α N-Spirosola-4, 6-dien-3-one(I). To a solution of solasodine (1g) in 100 ml of 20 % acetic acid was added dropwise a solution of potassium permanganate (1.2 g) in 150 ml of water with stirring in cold. The reaction mixture was stirred at room temperature for 18 hr. It was filtered and the darkish insoluble matter was repeatedly washed with hot 20 % acetic acid. The combined filterate and washings were ammoniated and extracted out with ethyl acetate. The ethyl acetate extract was washed dried (Na_2SO_4 anhydrous) and freed of the solvent. The residue was exhaustively extracted out with hot petroleum ether, when only a small quantity of insoluble matter remained, which was neglected. On removal of the solvent, the petroleum ether extract was taken up in minimum quantity of benzene-petroleum ether (1:1) in the hot. The crystallizate obtained on keeping the solution in cold formed fine needles melting at 180-81° (0.55 g, yield 55.5%). From spectral studies its structure corresponded to I. It is soluble in chloroform, ethyl acetate, methanol and sparingly soluble in ether, benzene and petroleum ether.

IR ν_{max} KBr (cm^{-1}): 1580, 1610, 1630, 1660, 3340; UV λ_{max} (nm): 283, shoulders at 208, 240; Mass *(m/e)*: 409.30026 (10 %) M^+ (Calculated for $C_{27}H_{39}NO_2$: 409.29805), 394, 381, 367, 296, 138 (100 %), 114, 55, 42.

Mother liquor of (I) was subjected to preparative thick-layer chromatography when a further compound could be isolated which was characterized as (25R)-6α-Hydroxy-22α N-spirosol-4-en-3-one (II) m.p. 124-125° (0.2 g, yield 19 %).

IR ν_{max} KBr (cm^{-1}): 1595, 1610, 1665, 3250, 3490; UV λ_{max} (nm): 240, shoulder at 207; Mass *m/e*: 427.3105 (4 %) M^+ (calculated for $C_{27}H_{41}NO_3$: 427.3085), 412 410, 399, 397, 152, 138 (100 %), 124, 114.

Acetylation of I – (25R)-N-Acetyl-22αN-Spirosola 4, 6-dien-3-one (III). III was obtained on refluxing I with acetic anhydride (1.5 mole) in pyridine for 1 hr and usual working. It melted at 120-121°.

IR ν_{max} $CHCl_3$ (cm^{-1}): 1600, 1630, 1640, 1670; UV λ_{max} (nm): 284, shoulder at 215; Mass *(m/e)*: 451 (2.3 %) M^+, 436, 408, 156, 180, 43 (100 %).

Acetylation of (II) – (25R)-N-Acetyl-3-oxo-22α N-Spirosol-4-en-6α Ol-Acetate (IV). (II) on acetylation with acetic anhydride (2.5 moles) and pyridine at room temperatue yielded the diacetyl product IV, m.p. 160-161°.

IR ν_{max} $CHCl_3$ (cm^{-1}): 1610, 1645, 1665, 1730; UN λ_{max} (nm): 240, shoulders at 205 and 225; Mass *(m/e)*: 511 (2 %) M^+, 481, 469, 468, 451, 55 (100 %).

Potassium Permanganate-Acetic Acid Oxidation of 3β-O-Acetyl-Solasodine – (25R)-7-Oxo-5α-22αN-Spirosolan-3β, 5α, 6α-Triol-3-Acetate (V). A solution of 0.3 g of potassium permanganate in 30 ml of water was added dropwise to a solution of 0.2 g of 3β-O-acetyl-solasodine in 20 ml of 20 % acetic acid with good cooling and stirring. The stirring was continued for two hr when the reaction mixture was filtered and washed with 20% acetic acid. The combined filterate and washings were ammoniated and the liberated base extracted out with ethyl acetate. The ethyl acetate layer was washed, dried (Na_2SO_4 anhydrous) and distilled under reduced pressure. The residue was exhaustively extracted out with hot petroleum ether and the petroleum ether insoluble portion was taken up in ether. On standing at room temperature overnight pet-ether and ether soluble fractions both gave whitish crystallizates, which on recrystallization from benzene and ether (1:1) formed aggregates of needles which melted at 150-51° (0.15 g, yield 67.8 %); from spectral studies it was characterised as V. It is soluble in chloroform, ethyl acetate, methanol and sparingly soluble in benzene, ether and petroleum ether.

IR ν_{max} $CHCl_3$ (cm^{-1}): 1595, 1715, 1730, 3300, 3510; UV λ_{max} (nm), 207, 230 Mass *(m/e)*: 503.3237 (2 %) M^+ (calculated for $C_{29}H_{45}NO_6$: 503.3246), 488, 460, 138, 114, 83, 43 (100%).

Acetylation of V – (25R)-N-Acetyl-7-Oxo-5α, 22αN-Spirosolan-3β, 5α, 6α-Triol-3,6-Diacetate (VI). Acetylation of V with acetic anhydride-pyridine at room temperature

and working up the reaction mixture in the usual manner afforded VI melting at 110-112°.

IR ν_{max} $CHCl_3$ (cm^{-1}): 1645, 1710, 1720, 1735, 3400; Mass *(m/e)*: 587 (2 %) M^+, 557, 545, 527, 512, 467, 43 (100 %).

Potassium Permanganate Oxidation of Solasodine in Pyridine – (25R)-3β, 5α, 6α-Trihydroxy-5α,22αN-Spirosolan-7-one (VII). To a solution of solasodine (0.3 g) in 15 ml of pyridine was added dropwise, a solution of potassium permanganate (0.4 g) in 45 ml of water under ice cooling. The reaction mixture was stirred in the cold for six hrs, extracted out with ethyl acetate. The crystalline residue left on working up the ethyl acetate extract in the usual way and removal of the solvent, afforded a uniform product which formed fine needles on recrystallisation with ethyl acetate melting at 189-90° (0.21 g, yield 62.7 %). It is soluble in chloroform, methanol, sparingly soluble in ethyl acetate, benzene and ether, insoluble in petroleum-ether. Its spectral data established its structure as VII.

IR ν_{max} KBr (cm^{-1}): 1620, 1715, 3250-3440 broad and strong band. UVλ_{max} (nm): 207, 230; Mass *(m/e)*: 461.3131 (3 %) M^+(calculated for $C_{27}H_{43}NO_5$: 461.3141), 433, 413, 138, 114 (100 %).

Acetylation of VII – (25R)-7-Oxo-5α-22αN-Spirosolan-3β,5α, 6α-Triol-3, 6-Diacetate (VIII). Acetylation of VII (0.1 g) was carried out in cold with acetic anhydride (1 ml) in pyridine. The diacetate VIII obtained on working up the reaction mixture in the usual manner crystallized out from methanol in slender rods m.p. 124-25°. It is insoluble in petroleum ether, ether, sparingly soluble in benzene and readily soluble in other bench solvents.

IR ν_{max} KBr (cm^{-1}): 1620, 1710-1730 broad and strong peak, 3400 broad; UV λ_{max} (nm): 215, 230; Mass *(m/e)*: 545.3327 (2%) M^+ (calculated for $C_{31}H_{47}NO_7$: 545.3352), 517, 502, 442, 138, 114 (100 %).

Hydrolysis of VIII – (25R)-3β, 6-Dihydroxy-spirosol-5-en-7-one (IX). VIII was taken up in methanolic potassium hydroxide (2 %) and left at room temperature for two hr. On working up the reaction mixture in the usual manner, a light yellow coloured solidish product was obtained, which showed one main UV active spot on TLC. After its purification through thick layer chromatography, it crystallised from methanol-benzene (1:1) in fine needles which melted at 138-40° and gave positive test with ferric chloride. Its spectral data established its structure as IX.

IR ν_{max} $CHCl_3$ (cm^{-1}): 1600, 1625, 1665, 3200-3500 broad and strong band; UV λ_{max} (nm): 214, 276; Mass *(m/e)*: 443.3026 (2 %) M^+ (calculated for $C_{27}H_{41}NO_4$: 443.3035), 428, 413, 299, 138, 114 (100 %).

(25R)-22,26-Acetyl-Epimino-7-Oxo-Cholest-22-en-3β, 5α, 6α, 16β-Tetraol-3, 6, 16-Triacetate (X). To a solution of VII (50 mg) in pyridine, excess of acetic anhydride was added and kept at room temperature for 36 hr. The product, obtained on working up the reaction mixture in the usual manner, crystallised out from methanol in fine needles, m.p. 100-101° and was characterized as X, IR ν_{max} $CHCl_3$ (cm^{-1}); 1640, 1660, 1710-1735 (broad peak), 3400; VU λ_{max} (nm): 215, 235; Mass *(m/e)*: 629.3552 (3 %) M^+ (calculated for $C_{35}H_{51}NO_9$: 629.3563), 587.3451 (calc. for $C_{33}H_{49}NO_8$: 587.3457), 572, 554, 544, 527, 512, 166 (100 %), 83, 55.

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