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Stereoisomeric Substituted 11-Keto-20-hydroxypregnanes. III

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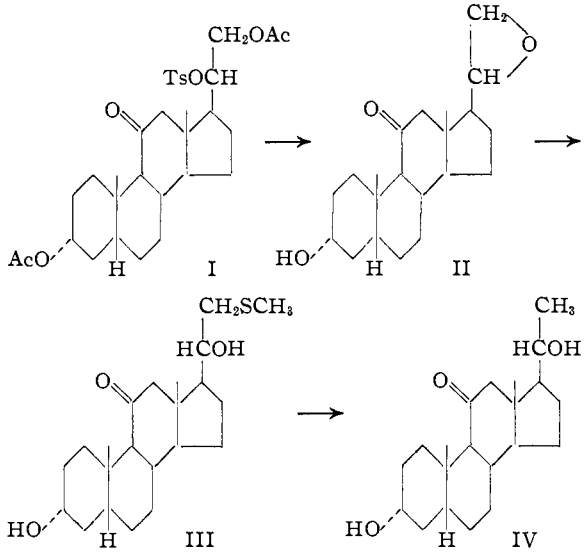
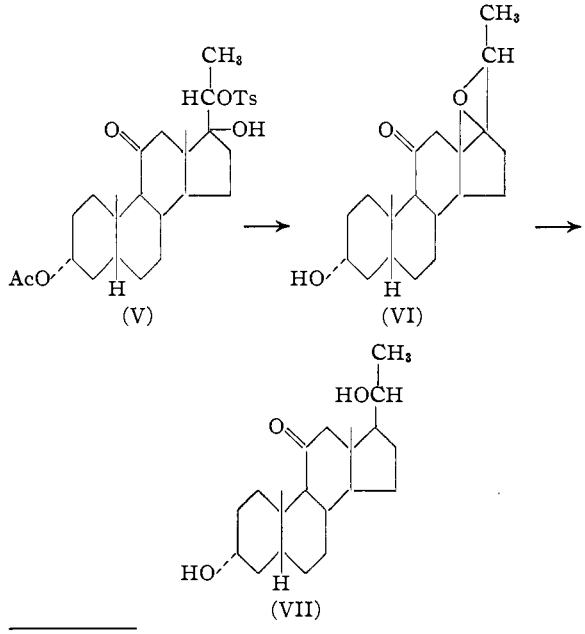
In Parts I and II[1,2] of this series, derivatives of 11-ketopregnane with the following side chain substituents have been described: 20-hydroxy (Class A), 20,21-dihydroxy (Class B), 17α,-20-dihydroxy (Class C) and 17α,20,21-trihydroxy (Class D). Within each class the 3α,20α- and 3α,20β-dihydroxypregnanes were linked with the corresponding 3-keto derivatives. Inasmuch as the arbitrary assignment of the α- and β-indices to a pair of 20-hydroxypregnanes in a given class and the repetition of this assignment at random to a pair in a second, a third and a fourth class is unappealing, the stereochemical correlation of the classes among themselves becomes desirable. The elucidation of this problem[3] forms the subject of Part III.

The reference pair for the four classes mentioned above may be conveniently selected from the simplest group, Class A. We designate the pregnane-3α,20-diol-11-one which melts at 237° as the 20β-isomer (VII) and its epimer (IV) as the 20α-form. In order to convert one of the two pregnane-3α,20,21-triol-11-one isomers into a 3α,20-diolone in such a manner that the relative configuration at C-20 might be known with certainty at all stages, a device which has been employed to prepare desoxy sugars was utilized.[4] A pregnane-3α,20,21-triol-11-one in the form of its 3,21-diacetate 20-tosylate (I)[1] was treated with aqueous alkali in acetone solution, giving the oxide (II). Since reactions of this kind proceed with inversion[5] on the carbon atom bearing the tosylate group the C-20 oxygen–carbon linkage is epimeric with that in the original triol. The oxide reacted smoothly with sodium methyl mercaptide to give the thioether (III) in which the configuration at C-20 remained unchanged, the attack of the methyl mercaptide ion having occurred at C-21. Direct treatment of the acetate tosylate with sodium methyl mercaptide also gave the 21-thioether in excellent yield. Evidently, the oxide is an intermediate.[6] Desulfurization[7] of III then gave pregnane-3α,20α-diol-11-one (IV). Hence the original triol is a member of the 20β-series.



The correlation of a member of Class C with the diolone reference pair followed a somewhat similar scheme. The 20-tosylate of one of the epimeric 3α,17α,20-triolones (V)[1] was converted to the oxide (VI), thus inverting the configuration at C-20. Reductive cleavage of the oxide appeared to proceed exclusively at C-17,[8] since



(1) Sarett, THIS JOURNAL, **71**, 1165 (1949).

(2) Sarett, *ibid.*, **71**, 1169 (1949).

(3) The stereochemical correlation of certain allopregnanetetrols of the 17β-hydroxy series with the corresponding triols has recently been accomplished by Salamon and Reichstein (*Helv. Chim. Acta*, **30**, 1616, 1929 (1947)).

(4) Jeanloz, Prins and Reichstein, *Helv. Chim. Acta*, **29**, 371 (1946).

(5) *Inter alia*, Philipps, *J. Chem. Soc.*, 44 (1923); 2565 (1925); Kenyon, Philipps and Taylor, *ibid.*, 173 (1933); Vischer and Reichstein, *Helv. chim. Acta*, **27**, 1332 (1944).

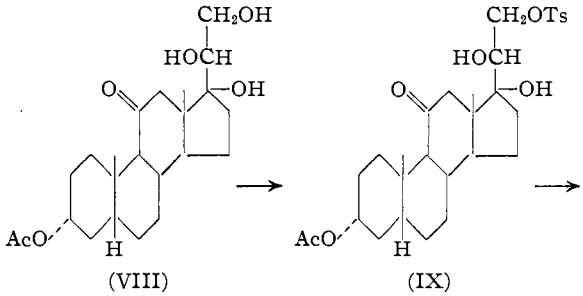
(6) The possibility of a dioxolone type of intermediate such as has been postulated by Winstein, Hass and Buckles (THIS JOURNAL, **64**, 2796 (1942)) in the solvolysis of 1,2-acetate tosylates appears unlikely, considering the strongly basic character of the reaction medium.

(7) Mozingo, Wolf, Harris and Folkers, THIS JOURNAL, **65**, 1013 (1943).

(8) *Cf.* Vischer and Reichstein, also Salamon and Reichstein, ref. 5.

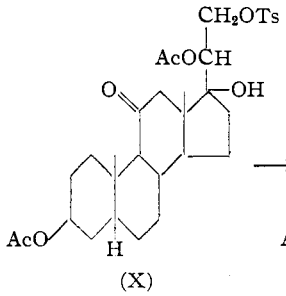
pregnane-3α,20β-diol-11-one was the sole product. This series indicated the C-20 hydroxyl group in the original triolone was alpha.

Finally, a pregnane-3α,17α,20,21-tetrol-11-one[2] was linked with one of the pregnane-3α,17α,20-triol-11-one epimers. The 3,20-diacetate 21-tosylate of the tetrolone (VIII) was prepared by hydrolysis of pregnane-3α,17α, 20,21-tetrol-11-one 3-acetate 20,21-acetonide followed by partial tosylation of the tetrolone 3-monoacetate (VIII). Since this derivative (IX) was not crystalline, the 20-hydroxy group was acetylated. Treatment of the tosylate diacetate (X) with sodium methyl mercaptide, followed by reacetylation of the triolone thioether gave the nicely crystalline diacetate (XI) in 89% yield.[9] With Raney nickel the 3α,-17α,20β-triolone diacetate (XII) was formed, from which it could be concluded that the parent tetrolone also was a 20β-hydroxypregnane.

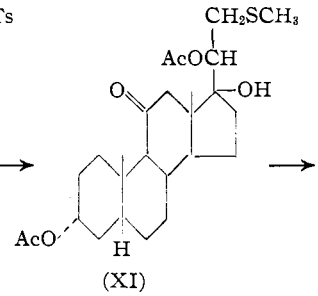


(VIII)

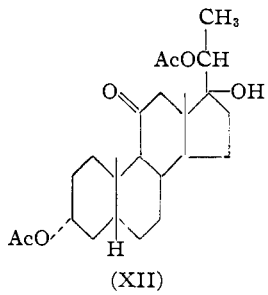
(IX)



(X)



(XI)



(XII)

Certain regularities are apparent from the results described above and in Parts I and II. Catalytic hydrogenation of an 11,20-diketopregnane gave predominantly one epimeric form of the 11-keto-20-hydroxypregnane,[10] the β-form according to our nomenclature. The quantity of α-isomer occurring as a by-product increased with the substitution of hydroxyl groups at the 17- and 21-positions but did not exceed about 20%. With osmium tetroxide Δ^{17}-11-ketopregnenes reacted to give glycols, the C-17 hydroxyl of which was invariably alpha[11] and the C-20 hydroxyl usually beta. The 17α,20α-glycol was obtained from a presumably geometrically isomeric Δ^{17}-pregnene. Confirming the results of other investigators,[12] we have found that hydroxylation of the 20,21-bond is less sterically selective, approximately equal amounts of both C-20 epimers being formed.

With the hope of finding a generally valid criterion for the diagnosis of configuration of the C-20 hydroxyl group, an examination of the molecular rotations of pairs of 11-keto-20-hydroxypregnane epimers was made. Although no particular regularity could be discerned, it presently became apparent that such a differentiation could be made on the basis of another type of rotational comparison. This consisted in the calculation of a characteristic increment or decrement in the molecular rotations of the 20β- and 20α-hydroxypregnanes, respectively, upon acetylation. That the "rule of shift"[13] could be applied to the steroids by analysis of such differences has been shown by Bernstein, Wilson and Wallis.[14] These workers demonstrated that the difference in molecular rotation of a 3β-hydroxystenol or stanol and its ester was constant and independent of the presence of subsidiary asymmetric centers or functional groups, providing that the latter were sufficiently distant from ring A to prevent the effects of vicinal action. Similarly, Plattner and Heusser[15] found in a study of partially acetylated cholic acid derivatives that the shifts of rotation caused by acetylation at the 3-, 7- and 12-positions were independent of each other. Barton in a series of papers[16] developed this approach (the "Method of Molecular Rotation Differences") and demonstrated its considerable versatility in the steroids and triterpenoids. This method of molecular rotation differences has been very recently applied to certain 20-hydroxypregnanes by Fieser and Fieser.[17] Since we have also carried out comparable calculations, with similar results, an elaboration of the excellent treatment given by these workers is superfluous.

(9) See remarks above on formation of thioether (III). Here, again the oxide is probably the intermediate, but whether or not this is the case, the configuration at C-20 must remain unaltered, since only the C-21 carbon–oxygen bond is broken.

(10) Marker, Kamm, Wittle, Oakwood, Lawson and Laucius (ref. 24) found that catalytic reduction of pregnane-3-ol-20-one's gave only one diol. For a discussion of Marker's system of nomenclature, see below.

(11) *Cf.*, however, Butenandt, Schmidt-Thomé and Paul, *Ber.*, **72**, 1112 (1939).

(12) *Inter alios*, Serini, Logemann and Hildebrand, *Ber.*, **72**, 391 (1939); Reich, Montigel and Reichstein, *Helv. Chim. Acta*, **24**, 977 (1941).

(13) Freudenberg, *Ber.*, **66**, 177 (1933).

(14) *J. Org. Chem.*, **7**, 103 (1942).

(15) Plattner and Heusser, *Helv. Chim. Acta*, **27**, 748 (1944).

(16) Barton, *J. Chem. Soc.*, 813 (1945); 512, 1116 (1946).

(17) Fieser and Fieser, *Experientia*, **4**, 285 (1948).

TABLE I

MOLECULAR ROTATIONS OF 11-KETO-20-HYDROXYPREGNANES AND DERIVATIVES

Class	Substituent at C-3	C-17	C-21	C-20	Molecular rotation,[a] degrees 20β	20α	Rotation difference,[b] degrees Δβ	Δα
A	HOα	Hα	H	HO	+160 (alc.)	+205 (alc.)		
	AcOα	Hα	H	HO	+250	...		
	HOα	Hα	H	AcO	...	+190		
	AcOα	Hα	H	AcO	+340	+255	+ 90	− 15
	O=	Hα	H	HO	+175	+230		
	O=	Hα	H	AcO	+280	+200	+105	− 30
B	HOα	Hα	HO	HO	+185 (alc.)	+190		
	AcOα	Hα	HO	HO	+285	+290		
	AcOα	Hα	AcO	HO	+280	+290		
	AcOα	Hα	AcO	AcO	+370	+235	+ 90	− 55
	O=	Hα	HO	HO	+215	+240		
	O=	Hα	AcO	AcO	+320	+195	+105	− 45
	O=, Δ⁴	Hα	HO	HO	+610	+610		
	O=, Δ⁴	Hα	AcO	AcO	+730	+570	+120	− 40
C	HOα	HOα	H	HO	+135	+ 80		
	AcOα	HOα	H	HO	...	+170		
	HOα	HOα	H	AcO	+215	+ 10	+80	− 70
	AcOα	HOα	H	AcO	+340	+105		
	O=	HOα	H	AcO	+290	+ 50		
	O=	HOα	H	HO	+165	+115	+120	− 65
D	HOα	HOα	HO	HO	+175 (alc.)	+160		
	AcOα	HOα	AcO	AcO	+505	+ 90		
	AcOα	HOα	AcO	HO	+300	...	+205	−160
	O=	HOα	AcO	AcO	+415	...		
	O=, Δ⁴	HOα	HO	HO	+505	...		
	O=, Δ⁴	HOα	AcO	AcO	+800	...	+220	...

[a] Molecular rotation = $M[\alpha]_D/100$ where M = molecular weight and $[\alpha]_D$ = specific rotation; values to nearest 5°.
[b] $\Delta\beta$ = molecular rotation of 20β-acetoxypregnane—molecular rotation of corresponding 20β-hydroxypregnane and similarly for Δ_α.

However, a short analysis of the rotational data of the 11-keto-20-hydroxypregnanes described in the present series of papers is not out of place.

Although it has been repeatedly demonstrated that the presence of neighboring asymmetric centers very seriously disturbs the constancy of a molecular rotation difference, we have attempted to apply the method to substituted 20-hydroxypregnanes in as quantitative a manner as possible. From an analysis of the rotational data in Table I, it appears that, independent of the presence of an oxygen function at C-21 or an α-hydroxyl group at C-17, the following rule is valid: In proceeding from the free 20-hydroxypregnane to the acetate, an increment in the molecular rotation may be observed in one of the configurational series while in the epimeric series a decrement is found. In addition, since the difference in rotation between the two 20-hydroxy-pregnanes[18] seems always to be less than the "spreading" effect produced by acetylation, the rotations of the 20-acetates of one configurational series are always greater than those of the other. In Table I, the column headed Δ_β gives the increment in molecular rotation observed upon acetylation of 11-keto-20-hydroxypregnanes having what we have called the β-configuration. Similarly Δ_α corresponds to the α-series. Although, considering all four classes, Δ_β and Δ_α vary in magnitude, within a given class their differences from the average do not lie far outside the experimental error.[19,20] Thus the relatively small fluctuation of Δ occurring with the introduction of one adjacent oxygen function is noteworthy. The cause of the great increase in Δ with the introduction of both adjacent oxygen functions (Class D) is not apparent.

Some sample calculations are given

Example 1. Pregnane-3α,20-diol-11-one
$\Delta\beta = (+340°) - (+250°) = +90°$
$\Delta\alpha = (+190°) - (+205°) = -15°$

Example 2. Pregnane-3α,20,21-triol-11-one
$\Delta\beta = (+370°) - (+280°) = +90°$
$\Delta\alpha = (+235°) - (+290°) = -55°$

Example 3. Pregnane-3α,17α-20,21-tetrol-11-one
$\Delta\beta = (+505°) - (+300°) = +205°$
$\Delta\alpha = +90° - [M]_D$ diacetate

(18) Pregnane is used here in a broad sense, to include allopregnane, Δ⁴-pregnene and the like.

(19) A difference of ±2° in the specific rotation is equivalent to an error of about ±8° in the molecular rotation and ±16° in Δ.

(20) The specific rotations taken in alcohol because of low solubility in acetone introduced a small additional error. In an effort to assess this quantity the rotations of five typical substituted pregnanes were measured in both solvents. Those in alcohol had the greater rotations by ±2, +4, +1, +3, and −1°. This small and inconstant difference (*cf.* Barton, Part III, ref. 16) was essentially within the experimental error and was not considered in calculating the values of Δ.

where [M]D diacetate is the molecular rotation of pregnane-3α,17α,20α,21-tetrol-11-one 3,21-diacetate. This quantity may be estimated by adding to the molecular rotation of the free tetrol, the contributions from acetylation at C-3 and C-21. The former, taken as the average of a number of rotation differences of 3α-hydroxy- and acetoxypregnanes is +90°.[21] If the latter be borrowed from a comparison of the rotations of a pair of 3,21-diacetates with the corresponding 3-monoacetates (Table I, Class B), according to which the rotation change produced by acetylation of a C-21 hydroxyl is negligible, the calculation may then be completed

$$\Delta_\alpha = (+90°) - [(+160°) + (+90°) + (0°)] = -160°$$

If the assumption is made that the rotational changes described above are at least qualitatively valid for 20-hydroxypregnanes[18] independent of the presence of an 11-keto group or C-3-hydroxyl configuration, a significant steric comparison may be made between this group of compounds and certain naturally occurring adrenal steroids. In particular, Reichstein's substance J, the C-20-hydroxyl of which has been arbitrarily defined as beta by Prins and Reichstein[22] and substance O, its C-20 epimer, may be linked with the present series. When this calculation is performed, it may be seen that J and O qualitatively follow the criteria for the 20β- and 20α-configurations, respectively. On this basis, the assignment of those 20-hydroxypregnanes which we have called beta to the same steric series as substance J has been made.

Allopregnane-3β,17α-20-triol (substance J)[23]

$$\Delta = (+100°) - (-25°) - (-15°) = +150°$$

Allopregnane-3β,17α,20-triol (substance O)[23]

$$\Delta = (-125°) - (-40°) - (-15°) = -70°$$

Another convention which has been used to designate epimeric 20-hydroxypregnanes is that of Marker and co-workers.[24] According to this system those 20-hydroxypregnanes occurring as urinary metabolites are given the alpha index and their epimers, produced in high yield by catalytic reduction of 20-ketopregnanes, the beta-designation. From the combined results of Butenandt and Schmide–Thomé[25] and Prins and Reichstein (ref. 23), who, in essence, converted substance J into the diol called according to Marker's nomenclature allopregnane-3β,20β-diol without disturbing the asymmetry at C-20, it is apparent that Marker's convention is also in agreement with those of Reichstein. In order further to test the rule of configuration described above, the rotation of the pregnane-3α,20-diols was compared with those of the corresponding diacetates.[26] Using the value [α]D+27°(alc.),[27] or [M]D+85°, for the rotation of the 3α,20α-diol (Marker's nomenclature) together with our experimental values for the rotations of the 3α,20β-diol, and the two diacetates, the two Δ values may be calculated

Pregnane-3α,20β-diol

$$\Delta = (+275°) - (+90°) - (+90°) = +105°$$

Pregnane-3α,20α-diol

$$\Delta = (+165°) - (+85°) - (+90°) = -10°.$$

These Δ's agree well with the Δ_β of +90° and the Δ_α of −15° found in the 11-ketodiol series. From these results it appears that the rotational rule rests on substantial ground.

Consideration of this rule reveals no *a priori* likelihood of its precise application to the 17β,20-dihydroxypregnanes, since the validity of the principal of optical superposition, upon which such a prediction must be based, is dubious in this system of adjacent asymmetric centers. That the "Rule of Shift," however, might find application in some manner not necessarily related to that in the 17α,20-dihydroxypregnane series, appears quite possible. Indeed, Reich, Montigel and Reichstein (ref. 12) have pointed out that in a series of three sterically correlated epimeric pairs of 17β,20-dihydroxypregnane derivatives, the fully acetylated compounds of the 20 "β"-group[28] all have the higher rotations. However, an examination of the rotations of all of the pertinent 17β-triols, tetrols and their acetates[29] shows that, unfortunately, no simple regularity based upon the "Rule of Shift" exists.

Experimental[30]

20α,21-Oxidopregnane-3α-ol-11-one (II).—A solution of 1.10 g. of pregnane-3α,20β,21-triol-11-one 3,21-diacetate 20-tosylate (I) m. p. 176–177°, in 35 cc. of 0.50 *N* methanolic potassium hydroxide was permitted to stand at 30° for two hours. Concentration of the solution *in vacuo* gave a crystalline precipitate which yielded after several recrystallizations from dilute acetone 400 mg. of the oxide; m. p. 173–174°, [α]^{25}D + 50°.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.58; H, 9.40.

Pregnane-3α,20α-diol-21-thiol-11-one Methyl Thioether (III)

A. From 20α,21-Oxidopregnane-3α-ol-11-one (II).—A solution of 183 mg. of the 20α,21-oxide, m. p. 173–174°, in 2.0 cc. of methanol containing 300 mg. of sodium methyl mercaptide was refluxed for one hour. The solution was diluted with water and extracted with ether. Concentration of the washed ethereal solution to dryness and recrystallization of the residue from a small volume of ether

(21) Plattner and Heusser (ref. 15) found +91° (alc.) and Barton (ref. 16) found +83° (all solvents).

(22) Prins and Reichstein, *Helv. Chim. Acta*, **23**, 1490 (1940).

(23) Calculated from data of Prins and Reichstein, ref. 22. The value for the conversion of the C-3 β-hydroxyl to acetate was taken as −15° (Barton, Part III, ref. 16).

(24) Marker, Kamm, Wittle, Oakwood, Lawson and Laucius, THIS JOURNAL, **59**, 2291 (1937).

(25) Butenandt and Schmide–Thomé, *Ber.*, **72**, 1960 (1939).

(26) We are indebted to Dr. K. Dobriner and to Dr. T. F. Gallagher of the Sloan-Kettering Institute for samples of these compounds.

(27) Beall, *Biochem. J.*, **31**, 35 (1937).

(28) The indices in the 17β,20-diol and 17β,20,21-triol series were assigned arbitrarily and, while they agree with each other (Salamon and Reichstein, ref. 3), they do not necessarily agree with those used in the 17α,20-diol and 17α,20, 21-triol series.

(29) Data collected by Salamon and Reichstein, ref. 3.

(30) Melting points were taken on the Kofler micro hot stage. Rotations were taken in acetone, c = 1.0, unless otherwise indicated.

yielded 171 mg. of the thioether, m. p. 147–148°. When the thioether was dissolved in a small volume of methanol, dense prisms of a solvated form quickly appeared, which lost solvent at *ca.* 140°, resolidified and remelted at 146–147°.

Anal. Calcd. for $C_{22}H_{36}O_3S$: C, 69.42; H, 9.54. Found: C, 69.46; H, 9.24.

B. From Pregnane-3α,20β,21-triol-11-one 3,21-Diacetate 20-Tosylate (I).—Treatment of 140 mg. of the tosylate with sodium methyl mercaptide in the manner described above and two crystallizations of the product from methanol yielded 60 mg. of the thioether (III), m. p. and mixed m. p. 141°, 147°.

Pregnane-3α,20α-diol-11-one (IV) from Pregnane-3α,-20α-diol-21-thiol-11-one Methyl Thioether (III).—To a suspension of 3 g. of Raney nickel in 25 cc. of 80% alcohol was added an alcoholic solution of 102 mg. of the thioether (III). The mixture was warmed on the steam-bath for ten minutes, filtered and concentrated to dryness *in vacuo*. The crystalline residue (65 mg.) was acetylated in the usual manner and the product oxidized with 50 mg. of chromic acid in 90% acetic acid to convert any 11-hydroxy compound to the 11-ketone. Addition of water to the acetic acid solution gave 60 mg. of crystals. After recrystallization from alcohol the 3α,20α-diolone diacetate had m. p. 235–237°. A mixed m. p. with an authentic sample showed no depression. Saponification of the diacetate gave pregnane-3α,20α-diol-11-one, m. p. and mixed m. p. 221°. A sample (15 mg.) of the diolone was oxidized with chromic acid (15 mg.) in 90% acetic acid and yielded pregnane-3,11,20-trione, m. p. and mixed m. p. 161–163°.

17α,20β-Oxidopregnane-3α-ol-11-one (VI).—To a solution of 350 mg. of pregnane-3α,17α,20α-triol-11-one 3-acetate 20-tosylate (V) in 6 cc. of acetone was added 4 cc. of 1 *N* aqueous potassium hydroxide. After two and one-half hours at room temperature the solution was concentrated *in vacuo* to a small volume, extracted with ether and the washed ethereal solution concentrated to dryness. The residue was dissolved in a small volume of methanol, water was added to turbidity and the solution then chilled to 0°. The difficultly crystalline hydrate of the oxide slowly separated. It decomposed (loss of solvent of crystallization) at about 102–105°. After several recrystallizations from cold dilute methanol, the crystals melted at 103–112°; $[\alpha]_D$ +54.5° (c = 0.5) (not corrected for water of crystallization). For analysis a sample was dried in a weighing pig at 110°.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.85; H, 9.70. Found: C, 75.76; H, 9.30. Loss in weight on drying: 2.2%.

Pregnane-3α,20β-diol-11-one (VII) from 17α,20β-Oxidopregnane-3α-ol-11-one (IV).—A solution of 30 mg. of 17α,20β-oxidopregnane-3α-ol-11-one in 50 cc. of alcohol with ¼ teaspoonful of Raney nickel was shaken in a bomb under hydrogen at 120° for two hours. The cooled solution was filtered, concentrated nearly to dryness *in vacuo* and the residue dissolved in acetone. The acetone solution was filtered to remove traces of inorganic matter, the solution concentrated to dryness and the residue crystallized from dilute alcohol. The diol melted at 236–237° and did not depress the m. p. of an authentic sample of pregnane-3α,20β-diol-11-one. Acetylation gave the 3α,-20β-diolone diacetate, m. p. and mixed m. p. 161.5–162.0°.

Pregnane-3α,17α,20β,21-tetrol-11-one 3-Acetate (VIII).—A solution of 600 mg. of pregnane-3α,17α,20β,21-tetrol-11-one 3-acetate 20,21-acetonide, m. p. 190–191°, in 1 cc. of warm acetic acid was treated with 1 cc. of water and the mixture heated on the steam-bath for fifteen minutes. Water was added and the suspension extracted with three 25-cc. portions of chloroform. The chloroform solution was washed with a little sodium carbonate solution, then with water and concentrated to dryness *in vacuo*. The tetrolone monoacetate was obtained as a white amorphous powder; yield, 471 mg.

Pregnane-3α,17α,20β-21-tetrol-11-one 3,20-Diacetate 20-Tosylate (X).—The 471 mg. of amorphous pregnane-3α,17α,20β,21-tetrol-11-one 3-acetate was dissolved in 2.0 cc. of pyridine and treated with 280 mg. of freshly recrystallized tosyl chloride (1.28 molecular equivalents). The solution was kept at room temperature overnight, then diluted with water and extracted with chloroform. The chloroform solution was washed successively with dilute hydrochloric acid, dilute sodium bicarbonate and water, then concentrated to dryness *in vacuo* at 20°. The amorphous residue was sparingly soluble in ether and weighed 671 mg. (103%). The amorphous 21-monotosylate (IX) was next converted to the 3,20-diacetate (X) by heating on the steam-bath with a mixture of 2.0 cc. of acetic anhydride and 1.5 cc. of pyridine for six minutes. The solution was cooled, and carefully diluted with water, giving 420 mg. of the crystalline diacetate tosylate. Recrystallization from chloroform–ether gave prisms, m. p. 145° (dec.). Another crystalline form, m. p. 140° (dec.), could be obtained by crystallization from methanol.

Anal. Calcd. for $C_{32}H_{44}O_9S$: C, 63.55; H, 7.33. Found: C, 63.27; H, 7.52.

Pregnane-3α,17α,20β-triol-21-thiol-11-one 3,20-Diacetate Methyl Thioether (XI).—A solution of 200 mg. of the tosylate (X), m. p. 145° (dec.), in 4 cc. of methanol containing 600 mg. of sodium methyl mercaptide was refluxed for one hour. Dilution with water and removal of the methanol *in vacuo* gave hydrated crystals which could not be recrystallized from organic solvents. These crystals were combined with the amorphous product obtained by extraction of the mother liquors with chloroform, giving 160 mg. of crude reaction product, which contained some extraneous sulfur compounds. This material was acetylated with pyridine–acetic anhydride in the usual manner and dilution of the reaction mixture with water then gave 140 mg. (89%) of the diacetate, m. p. 191–194°. Recrystallization from methanol gave a product melting at 194–196°.

Anal. Calcd. for $C_{26}H_{40}O_6S$: C, 64.98; H, 8.39. Found: C, 65.18; H, 8.42.

Pregnane-3α,17α,20β-triol-11-one Diacetate (XII) from Pregnane-3α,17α,20β-triol-21-thiol 3,20-Diacetate Methyl Thioether.—A solution of 28 mg. of the thioether (XI) in 10 cc. of alcohol was refluxed with 0.5 teaspoonful of Raney nickel for ten minutes, the nickel removed by centrifugation, and the solvent removed *in vacuo*. The crystalline residue was dissolved in ether and recrystallized by concentration. The product, which melted at 233–245°, was best separated from a small amount of high melting impurity (11-hydroxy analog(?)) by chromatography over 3 g. of alumina. The eluates obtained with 1:1 ether–petroleum ether through ether were combined and recrystallized from a small volume of ethyl acetate. The pregnane-3α,17α,20β-triol-11-one diacetate then had m. p. 248–249°. A mixed melting point with an authentic sample (m. p. 249–250°) showed no depression.

Rotations of Pregnanediols.—Pregnane-3α,20α-diol, +27° (alc.); pregnane-3α,20β-diol (m. p. 239°), +25° (alc.); pregnane-3α,20α-diol diacetate (m. p. 180.5–182.0°), +41°; pregnane-3α,20β-diol diacetate (m. p. 112–113°), +68°.

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Summary

The stereochemical correlation of the 11-ketopregnane-3α,20,21-triols, the 11-ketopregnane-3α,-17α,20-triols, and the 11-ketopregnane-3α,17α,20,-21 tetrols with the 11-ketopregnane 3α,20-diols is

described. Analysis of the rotations of various 11-keto-20-hydroxypregnanes by the "Method of Molecular Rotation Differences" shows certain regularities independent of the presence of oxygen functions at the C-17 or C-21 positions.

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Senecio Alkaloids: α- and β-Longilobine from *Senecio longilobus*

BY ROGER ADAMS AND T. R. GOVINDACHARI

The investigations[1] on *Senecio* alkaloids, of which nearly forty have been isolated so far, have brought to light the striking similarities of many members belonging to this group. Essentially, most of these alkaloids are alkamine esters, in which a pyrrolizidine base carrying two hydroxyls is esterified with aliphatic acids either on one or both hydroxyls. While the heterocyclic component has proved to be retronecine in a large number of cases, the acid fragments exhibit a bewildering variety. These necic acids, although many of them are isomeric, differ widely in melting point, optical rotation, etc., and only in a few cases has the acid moiety been found to be common to two different alkaloids.

The work presented here comprises an investigation of the alkaloids of *Senecio longilobus*, a plant to which a large percentage of the West Texas cattle losses may probably be attributed rather than to the loco plant. Manske[2] was the first to isolate a product from this source which he designated as longilobine. It became apparent very early in the course of the work that longilobine was not a single entity, but a mixture of at least two components. It has been known for a long time that many species of *Senecio* elaborate more than one alkaloid and several alkaloids have been isolated from the same species by fractional crystallization procedures.[3,4] In some instances, however, the attempts at separation of such mixtures have failed completely.[5,6]

Manske has reported longilobine to be $C_{18}H_{23}O_5N$ and the acid obtained by hydrolysis $C_{10}H_{14}O_5$, although his analytical data did not agree satisfactorily with these empirical formulas. The analysis of a sample of longilobine prepared in this Laboratory suggested the same formula as proposed by Manske but likewise was not as close to the theoretical as might be expected of a pure product. In spite of several crystallizations a constant melting point and specific rotation were not reached and the values obtained did not coincide with those reported by Manske.

It has been suggested[3a,6] that these alkaloids are difficult to burn and often gave low values for carbon. It seemed more likely that some other alkaloid with lower carbon content was associated with the $C_{18}H_{23}O_5N$ alkaloid and that this could not be effectively removed by crystallization.

The use of chromatographic adsorption methods for the separation and purification of alkaloids has been a well-established procedure but no application of chromatography has been made so far in the case of the *Senecio* alkaloids. The chromatography of colorless substances presents a difficult problem and many ingenious ways have been devised to locate the invisible zones.[7] Mention may be made of the use of the ultraviolet lamp,[8] the brush method,[9] the use of fluorescent adsorbents[10] and the use of total reflection on a thick glass plate[11] for the detection of boundaries.

They proved unsatisfactory for the separation of the constituents of the crude alkaloid from *Senecio longilobus* for various reasons. Preliminary tests indicated that the "brush method" might have been successfully applied if a further careful study had been undertaken. Investigations along these lines were abandoned when it was found that two main fractions corresponding to $C_{18}H_{23}O_5N$ and $C_{18}H_{25}O_6N$ could be separated easily on a column of alumina by a simple empirical procedure. The criterion of purity was set as a constant optical rotation, which did not change or lead to fractions having higher and lower optical rotations on further adsorption and fractional elution. The liquid chromatogram method, combined with the examination of the column cut into portions arbitrarily, proved sufficiently useful for the purpose.

An initial treatment of the crude alkaloid with alumina was used for effecting a preliminary separation of products before the usual chromatographic procedure. Longilobine with a specific

(1) For a detailed discussion, the following papers may be consulted: (a) Adams and Rogers, THIS JOURNAL, **61**, 2815 (1939); (b) Adams, Hamlin, Jelinek and Phillips, *ibid.*, **64**, 2760 (1942); (c) Adams, Carlin and Rogers, *ibid.*, **64**, 571 (1942). The authors also had access to a chapter on *Senecio* alkaloids contributed by Dr. N. J. Leonard, University of Illinois, to a forthcoming monograph on alkaloids, under the editorship of Dr. R. H. F. Manske.

(2) Manske, *Can. J. Res.*, **17B**, 1 (1939).

(3) (a) de Waal and Onderstepoort, *J. Vet. Sci. Animal Ind.*, **16**, 149 (1941); (b) **12**, 155 (1939).

(4) Barger and Blackie, *J. Chem. Soc.*, 584 (1937).

(5) Barger and Blackie, *ibid.*, 743 (1936).

(6) Manske, *Can. J. Res.*, **14B**, 8 (1936).

(7) Zechmeister, *Annals New York Academy of Sciences*, **49**, 149 (1948).

(8) Karrer and Schöpp, *Helv. Chim. Acta*, **17**, 693 (1934); Winterstein and Schön, *Z. physiol. Chem.*, **230**, 139 (1939).

(9) Zechmeister, Cholnoky and Ujhelyi, *Bull. soc. chim. Biol.*, **18**, 1885 (1936).

(10) Brockmann and Volpers, *Ber.*, **80**, 77 (1947).

(11) Claesson, *Nature*, **159**, 708 (1947).