# The Oxidation of Steroidal Amines to Nitro Steroids[1]

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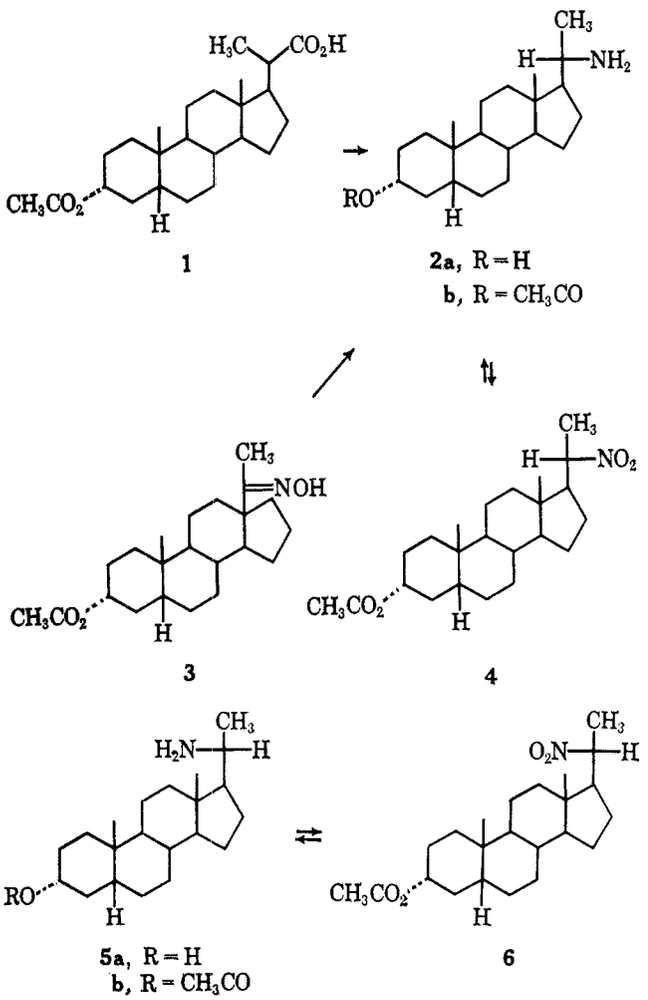
The oxidation of steroidal 3α-, 3β-, 20α- and 20β-amines to the corresponding nitro compounds, using *m*-chloroperbenzoic acid, is described. The conversion $R_1R_2{}^*CHNH_2$ to $R_1R_2{}^*CHNO_2$ gives products which retain stereochemical integrity at the asymmetric carbon. Optical rotatory dispersion and circular dichroism data have been obtained for the 20-nitro compounds. The equilibration of epimeric 20-nitro steroids leads to a 3:2 ratio of 20β:20α isomers.

In connection with a synthetic problem we required the epimeric 20-nitro steroids **4** and **6**. Two possible routes, starting from the C-20 oxime **3** or the C-20 amines **2b** and **5b**, were considered (Scheme I). The

SCHEME I



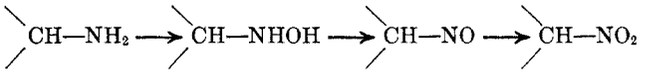

first would involve conversion of the C-20 oxime **3** to the 20-nitro compounds using either per acid[2] or the modified Iffland[3] procedure (introduced by Patchett, *et al.*[4]), using N-bromosuccinimide oxidation to the *gem*-bromonitro compound, followed by borohydride reduction to the nitro compound.

An alternative pathway would consist in the oxidation of the epimeric 20-amines with per acid. Emmons[5] has described the conversion of a number of amines to nitro compounds, in reasonable yield, using anhydrous peracetic acid (acetic anhydride–90% hydrogen peroxide). These conditions seemed rather vigorous for our purpose, and no oxidations of amines attached to asymmetric carbon atoms were cited by Emmons. However, we found one report[6] describing the oxidation of a 17β-amino steroid to the 17β-nitro steroid in about 50% yield, using perbenzoic acid.

Of the two routes, that starting from the oxime, although seemingly more direct, appeared likely to give mixtures of the epimeric 20-nitro compounds. The conversion[4] of a steroidal 17-oxime to only the 17β-nitro compound is not unexpected, as this would surely be the result of both thermodynamic and kinetic control.

Nevertheless, we treated the 20-oxime **3** with N-bromosuccinimide in dioxane containing potassium hydrogen carbonate, but obtained complex mixtures, containing modest amounts of nitro compound as shown by the infrared spectra. Other attempts, using modified conditions and in all cases monitoring the reaction by thin layer chromatography, gave equally complex mixtures. Discouraged by this, we did not study the action of per acid on the oxime, but went straight to the per acid oxidation of the 20-amines **2b** and **5b**.

The 20α-amine **2b** was readily available through the Curtius degradation of 3α-acetoxybisnorcholanic acid (**1**).[7] The procedure described by Julian, *et al.*,[8] proved to be excellent, giving the amine **2b** in 90% yield. Fearing that the conditions employed by Emmons might lead to mixtures of epimeric nitro compounds, we used *m*-chloroperbenzoic acid in chloroform as the oxidizing system. It seems very probable that the sequence of events is[5,9]




(1) This investigation was supported by Public Health Service Grants AM-07786 and GM-1183.

(2) W. D. Emmons and A. S. Pagano, *J. Am. Chem. Soc.*, **77**, 4557 (1955).

(3) D. C. Iffland and G. X. Criner, *ibid.*, **75**, 4047 (1953).

(4) A. A. Patchett, F. Hoffman, F. F. Giarusso, H. Schwam, and G. E. Arth, *J. Org. Chem.*, **27**, 3822 (1962).

(5) W. D. Emmons, *J. Am. Chem. Soc.*, **79**, 5528 (1957).

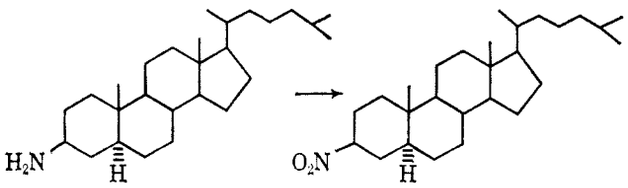
(6) L. N. Nysted and R. R. Burtner, *J. Org. Chem.*, **27**, 3175 (1962).

(7) J. Sawlewicz and T. Reichstein, *Helv. Chim. Acta*, **20**, 949 (1937).

(8) P. L. Julian, E. W. Meyer, and H. C. Printy, *J. Am. Chem. Soc.*, **70**, 887 (1948).

(9) N. Kornblum, R. J. Clutter, and W. J. Jones [*ibid.*, **78**, 4003 (1956)] proposed such a pathway in the conversion of $R_3C{-}NH_2$ to $R_3C{-}NO_2$ using potassium permanganate as the oxidizing agent.

SCHEME II

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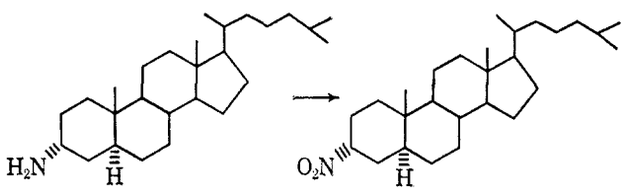


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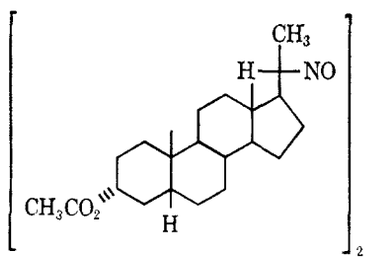


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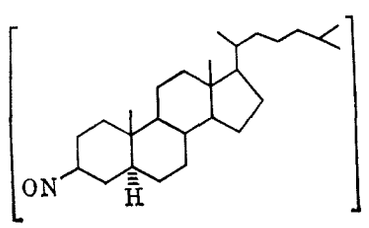


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The reaction was initially carried out using 3.3 moles of per acid/mole of amine (**2b**), in chloroform containing calcium carbonate. A mixture resulted, containing about 50% of nitro compound (as judged by the infrared absorption at 1560 cm.$^{-1}$ shown by the mixture) and another product which from the ultraviolet absorption[10,11] ($\lambda_{max}^{heptane}$ 298 m$\mu$) seemed to be the 20-nitroso dimer **11** (Scheme II). The infrared spectrum of this substance showed absorption at 1190 cm.$^{-1}$, characteristic of *trans*-nitroso dimers[10] which show a band in the range 1176–1290 cm.$^{-1}$. There is precedent[11] for the conversion of amines to nitroso dimers by partial oxidation. The conditions were then modified so that the amine was added slowly to a solution of the per acid (7 moles/mole of steroid) in refluxing chloroform. In this way it was hoped that accumulation (and subsequent dimerization) of nitroso compound could be prevented. Indeed, under these conditions a 58% yield of the crystalline 20$\alpha$-nitro compound **4** was obtained, showing the correct analysis and strong infrared absorption[12] at 1560 cm.$^{-1}$. It was then necessary to prepare the 20$\beta$ isomer **6** and compare the properties of the two compounds.

The 3$\alpha$-acetoxy-20$\beta$-amine **5b** was prepared by a standard route. Thus, the 20-oxime **3**, on reduction with sodium in 1-propanol, gave a mixture of 20-amines **2a** and **5a**, which were readily resolved on thin layer chromatography using a chloroform–methanol–ammonia system (see Experimental Section). The ratio of 20$\beta$:20$\alpha$-amine appeared to be roughly 3:2. Such mixtures are hard to separate by conventional chromatography. Thus Nedelec,[13] who recently prepared the amines **2a** and **5a**, used a circuitous route to avoid such a separation. This author formed the Schiff base from 3$\alpha$-hydroxy-5$\beta$-pregnan-20-one and benzylamine, reduced with sodium borohydride, separated the resulting 20-N-benzylamino compounds by chromatography, and then hydrogenolyzed the benzyl groups.

However, we decided to chromatograph our mixture of amines **2a** and **5a**, using the technique recently described by Duncan.[14] This involves column chromatography on silica gel, using a ratio of about 800:1 of adsorbent to mixture and the same elution system as is found suitable for resolution on thin layer chromatography. By using a fraction collector this method is, in our experience, more convenient than thick layer chromatography for difficult separations on the 500-mg. to 2-g. scale.

In the case at hand, we found that the amines also separated on thin layer chromatography on alumina, using a chloroform–methanol–diethylamine mixture. Chromatography on alumina by the Duncan technique, using this eluting system and a fraction collector, resolved the 20-amine mixture quite cleanly (see Experimental Section), giving first 54% of pure 20$\beta$-amine **5a** followed by 32% of pure 20$\alpha$-amine **2a** after a few fractions containing mixtures. The 20$\alpha$-amine was shown to be identical with the product obtained by hydrolysis of the 3$\alpha$-acetoxy-20$\alpha$-amine **2b**.

The 3$\alpha$-hydroxy-20$\beta$-amine **5a** was then acetylated selectively at C-3 in essentially quantitative yield using acetic anhydride–acetic acid–hydrochloric acid, and the resulting 3$\alpha$-acetate **5b** was oxidized with *m*-chloroperbenzoic acid as described previously. The desired 20$\beta$-nitro compound **6** was obtained in 79% yield.

Although the 20-nitro compounds **4** and **6** showed the same $R_f$ values on thin layer chromatography, they could be separated by gas chromatography. Using a 1% QF-1 column (8 ft.) at 235° and argon flow rate of 80 cc./min., compounds **4** and **6** showed retention times of 28.7 and 25.0 min., respectively. (For retention times relative to cholestenone, and details of the g.l.p.c., see Experimental Section.) Although complete separation to the base line was not achieved, the separation permitted the conclusion that the nitro compounds each contained about 5% of the other isomer. The oxidation of the amines had therefore resulted in substantially complete retention of configuration at C-20 in the resulting nitro compounds.

Further, the g.l.p.c. results allowed at least an approximate determination of the equilibrium mixture of the C-20 nitro compounds. Thus, using sodium bi-

(10) B. G. Gowenlock and W. Lüttke, *Quart. Rev.* (London), **12**, 321 (1958).
(11) W. D. Emmons, *J. Am. Chem. Soc.*, **79**, 6522 (1957).
(12) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., Methuen and Co. Ltd., London, 1958, p. 299.
(13) L. Nedelec, *Bull. Soc. Chim. France*, 557 (1963).
(14) G. R. Duncan, *J. Chromatog.*, **8**, 37 (1962).

carbonate in 95% ethanol at reflux, the equilibrating conditions used by Zimmerman and Nevins[15] for a phenyl-substituted nitrocyclohexane, the equilibrium mixture of 20$\beta$:20$\alpha$ was 3:2. With tetramethylguanidine in boiling toluene, the 20$\beta$:20$\alpha$ ratio was also 3:2. (For details, see Experimental Section.) The bicarbonate equilibration results lead to a calculated free-energy change of 0.28 kcal./mole.

It is interesting to note that sodium–alcohol reductions of steroidal 20-oximes give 20-amine mixtures in which the 20$\beta$ isomer predominates.[16] Although variable ratios of 20$\beta$:20$\alpha$ isomers have been reported, the general similarity of these ratios to the equilibration data for the 20-nitro compounds suggest that the products from such reductions reflect thermodynamic control.

The n.m.r. spectra[17,18] of **4** and **6** showed the C-18 protons resonance at 0.68 and 0.76 p.p.m., respectively, while the C-21 methyl protons appeared as doublets ($J$ = 6.5 c.p.s.) centered on 1.54 (compound **4**) and 1.47 p.p.m. (compound **6**).

The optical rotatory dispersion and circular dichroism data[19] for compounds **4** and **6** are shown in Table I.

TABLE I

Optical Rotatory Dispersion[a]

| | [$\Phi$], deg. | $\lambda$, m$\mu$ | [$\Phi$], deg. | $\lambda$, m$\mu$ |
|---|---|---|---|---|
| 20$\alpha$-Nitro (**4**) | −1000 (tr.) | 333 | +4170 (pk.) | 258 |
| 20$\beta$-Nitro (**6**) | +1000 (pk.) | 333 | + 140 (tr.) | 286 |

Circular Dichroism[b]

| | $\lambda$, m$\mu$ | $\Delta\epsilon$ | [$\theta$] × $10^{-3}$ |
|---|---|---|---|
| 20$\alpha$-Nitro (**4**) | 291 (min.) | −1.03 | −3.4 |
| 20$\beta$-Nitro (**6**) | 307 (max.) | +0.23 | +0.74 |
| | 249 (min.) | −0.17 | −0.55 |

[a] Measured using a Bellingham and Stanley-Bendix-Ericsson automatic recording spectropolarimeter, Polarmatic 62; methanol solutions; tube length, 0.1 dm.; temperature, 20–25°; and concentrations of 2.095 mg./ml. and 0.584 mg./ml. for compounds **4** and **6**, respectively. [b] Measured using the Roussel-Jouan Dicrograph; methylcyclohexane–isopentane (1:3) solutions at 20°, concentrations of 1.29 mg./2.08 g. and 1.23 mg./1.68 g. for **4** and **6**, respectively.

Finally, reduction of the nitro compounds **4** and **6** with lithium aluminum hydride in boiling dioxane gave complex mixtures in each case. However, the 20$\alpha$-amine **2a** was shown by t.l.c. to be a major product from **4**, as was the 20$\beta$-amine **5a** from the 20$\beta$-nitro compound **6**. In each case minor amounts of the epimeric 20-amines were present, which could be readily accounted for by the small amount of epimeric contaminant in each nitro compound. Epimerization[20] at C-20 during the reaction must therefore be minor in extent.

(15) H. E. Zimmerman and T. W. Nevins, *J. Am. Chem. Soc.*, **79**, 6559 (1957).

(16) See, for example, P. L. Chien, W. E. McEwen, A. W. Burgstahler, and N. T. Iyer, *J. Org. Chem.*, **29**, 315 (1964).

(17) Measured on a Varian A-60 spectrometer, using deuteriochloroform as solvent and tetramethylsilane (TMS) as internal reference. Chemical shifts are given in $\delta$ units (*i.e.*, TMS = 0).

(18) The n.m.r. spectra provided independent confirmation of the stereochemistry at C-20, for reasons which will be discussed in a forthcoming publication by C. H. Robinson and P. Hofer.

(19) We are greatly indebted to Professor W. Klyne (London) for the O.R.D. data and to Dr. G. Snatzke (Bonn) for the C.D. data.

(20) N. Kornblum and L. Fishbein [*J. Am. Chem. Soc.*, **77**, 6266 (1955)] have observed that hydride reduction of 2-nitrooctane results in racemic 2-aminooctane.

In order to examine the scope of this oxidative procedure for conversion of $R_1R_2$*$CHNH_2$ to $R_1R_2$*$CHNO_2$ with retention of configuration at the asymmetric carbon, we turned to the known[21] 3$\alpha$- and 3$\beta$-aminocholestanes (**7** and **9**), as suitable examples of substituted cyclohexylamines.

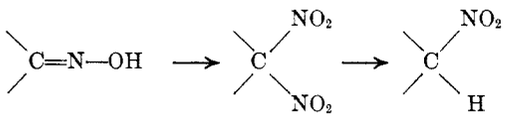
Oxidation of the 3$\beta$-amine **7** with *m*-chloroperbenzoic acid, under the conditions described above, gave pure 3$\beta$-nitro-5$\alpha$-cholestane (**8**) in 45% yield (Scheme II). In addition, the 3-nitroso dimer **12** was isolated. The structure of the latter followed from the analytical data, the ultraviolet absorption ($\lambda_{max}^{heptane}$ 294 m$\mu$), and infrared absorption at 1180 cm.$^{-1}$ (*trans* dimer). This compound proved difficult to purify by crystallization, owing to rearrangement to cholestanone oxime.

3$\alpha$-Amino-5$\alpha$-cholestane (**9**) was prepared by lithium aluminum hydride reduction of 3$\alpha$-azido-5$\alpha$-cholestane in the manner described by Henbest and Jackson.[22] In passing, we note that, although Henbest and Jackson prepared the 3$\alpha$-azide from 3$\beta$-*p*-toluenesulfonoxy-5$\alpha$-cholestane by the action of sodium azide in dimethylformamide at 153° for 20 hr., we found the reaction to be complete after 5 min. at 202°, using sodium azide in N-methylpyrrolidone.

Oxidation of the 3$\alpha$-amine **9** with *m*-chloroperbenzoic acid gave, in 28% yield, the 3$\alpha$-nitro compound **10**.

At this stage in the work, we became aware of work by Bull, *et al.*,[23] describing the synthesis of a number of nitro steroids, including the 3$\alpha$- and 3$\beta$-nitro compounds **8** and **10**. We refrain, therefore, from further comment on these two nitro compounds, except to note that the physical data are in full accord with those given by the above authors.

These authors employed the sequence shown below.




For the preparation of the *gem*-dinitro intermediate, fuming nitric acid and 30% hydrogen peroxide were employed, and the *gem*-dinitro compound was then hydrogenolyzed to the mononitro compound. This procedure gave at C-4 and C-6 the $\beta$ isomer, at C-7 the $\alpha$ isomer, and at C-3 a mixture of $\alpha$ and $\beta$ isomers. Equilibration of these nitro compounds then gave the more stable epimers.

We believe the procedure described in this paper represents a more convenient method because of the less hazardous reagent used and because it is a one-step reaction *vs.* a two- or three-step sequence. Further, in the only cases we have for comparison, the yields of 3$\beta$-nitro-5$\alpha$-cholestane (45%) and 3$\alpha$-nitro-5$\alpha$-cholestane (28%) reported in this paper compare very favorably with the over-all yields of 29 and approximately 4%, respectively, reported by Bull, *et al.*[23]

Furthermore, axial amines can be obtained readily by the sequence *p*-toluenesulfonate → azide → amine, while equatorial amines generally result as the predominant products from sodium–alcohol reduction of oximes.

(21) C. W. Shoppee, D. E. Evans, H. C. Richards, and G. H. R. Summers, *J. Chem. Soc.*, 1649 (1956).

(22) H. B. Henbest and W. R. Jackson, *ibid.*, 954 (1962).

(23) J. R. Bull, E. R. H. Jones, and G. D. Meakins, *ibid.*, 2601 (1965).

## Experimental Section[24]

**3α-Acetoxy-20α-amino-5β-pregnane (2b) from 3α-Acetoxybisnorcholanic Acid.**—3α-Acetoxybisnorcholanic acid (10.0 g.) was dissolved in oxalyl chloride (15 ml.) at room temperature, with swirling. After 15 min., the solution was evaporated to dryness *in vacuo*, and the residue was dissolved in benzene and again taken to dryness. The resulting crude acyl chloride was dissolved in acetone (180 ml.), and sodium azide (3.2 g.) was added to the stirred cooled (0–5°) solution. After 15 min. the solution was poured into ice–water (300 ml.), and the precipitated acyl azide was filtered off, washed with water, and added to 200 ml. of glacial acetic acid and 30 ml. of water. The mixture was slowly warmed to 70° until solution was obtained and was then left for 1 hr. at 70°. The solution was now evaporated *in vacuo*, and the resulting oil was dissolved in chloroform. The chloroform solution was washed with 2 *N* sodium hydroxide solution, then with water, and evaporated to give the 3α-acetoxy-20α-amine **2b** (8.85 g.). This crude product was shown to be essentially pure by t.l.c.[25] A small sample recrystallized from ether–petroleum ether (b.p. 40–60°) showed m.p. 131–137°, $[\alpha]_D$ +30°.

*Anal.* Calcd. for $C_{23}H_{39}NO_2$: C, 76.40; H, 10.87; N, 3.87. Found: C, 75.78; H, 10.86; N, 3.94.

The amine **2b** was further characterized by conversion to 3α-hydroxy-20α-acetamido-5β-pregnane by acetylation (pyridine–acetic anhydride at room temperature for 18 hr.) followed by selective hydrolysis at C-3 using 2% methanolic potassium hydroxide at room temperature for 18 hr. The amide showed m.p. 230–231° (from acetone), $[\alpha]_D$ +8°.

*Anal.* Calcd. for $C_{23}H_{39}NO_2$: C, 76.40; H, 10.87; N, 3.87. Found: C, 76.64; H, 10.86; N, 3.82.

**3α-Hydroxy-20α-amino-5β-pregnane (2a) and the 20β Isomer (5a) by Sodium–Propanol Reduction of the 20-Oxime (3).**—3α-Acetoxy-5β-pregnane-20-one was converted to the 20-oxime **3** in the usual manner (hydroxylamine hydrochloride–sodium acetate in 95% ethanol under reflux for 18 hr.) and showed double m.p. 107–110° and 122–123°, $[\alpha]_D$ +52°.

*Anal.* Calcd. for $C_{23}H_{37}NO_3$: C, 73.56; H, 9.93; N, 3.73. Found: C, 73.67; H, 9.85; N, 3.91.

A solution of the oxime **3** (700 mg.) in 1-propanol (30 ml.) was brought to reflux, and sodium metal (1.50 g.) was added, in small pieces, over a period of 2 hr. The solution was then concentrated *in vacuo* to about 10 ml., diluted with water, and extracted with chloroform. The crude amine mixture was shown by t.l.c.[25] to contain the two amines **2a** and **5a** in the ratio of about 2:3. The amines also separated on alumina plates, using chloroform containing 3% methanol and 0.75% diethylamine. The mixture was then chromatographed according to Duncan[14] on alumina (Alcoa, 560 g.), using chloroform containing 3% methanol and 0.75% diethylamine as eluent. Sudan red II (0.5 mg.) was applied to the column, followed by the amine mixture, using the chloroform–methanol–diethylamine mixture as solvent in each case. After the dyestuff had been eluted from the column, 20-ml. fractions were taken, using an automatic fraction collector. The first fractions containing solid were combined to give, after evaporation, pure (by t.l.c.) 3α-hydroxy-20β-amino-5β-pregnane (**5a**, 380 mg.). This compound showed m.p. 176–177° (from benzene), $[\alpha]_D$ 0° [lit.[13] m.p. 169°, $[\alpha]_D$ +12° (methanol)].

After a few fractions containing mixtures, the pure 20α-amine **2a** (225 mg.) was obtained. This compound showed m.p. 201–203° (from benzene), $[\alpha]_D$ +20° [lit.[13] m.p. 209°, $[\alpha]_D$ +25° (methanol)].[26] The same 20α-amine **2a** was obtained by hydrolysis of the 3α-acetoxy-20α-amine **2b** using 5% methanolic potassium hydroxide at room temperature for 40 hr. (identity confirmed by melting point, mixture melting point, infrared, and t.l.c.).

**3α-Acetoxy-20β-amino-5β-pregnane (5b).**—The 3α-hydroxy-20β-amine **5a** (284 mg.) was added to glacial acetic acid (1.6 ml.) and concentrated hydrochloric acid (2.4 ml.), and the suspension was stirred and cooled with ice while acetic anhydride (6 ml.) was added slowly. Stirring was continued for 24 hr. when the clear solution was concentrated to low volume *in vacuo*, diluted with water, and extracted twice with ether. The aqueous phase was then basified with 2 *N* sodium hydroxide solution and extracted with chloroform. The chloroform layer was washed with water and evaporated to give the 3α-acetoxy-20β-amine **5b** (300 mg.). This material was homogeneous by t.l.c.,[25] and the infrared spectrum showed bands at 1735 and 1260 cm.$^{-1}$ (acetate) but no absorptions due to the amide grouping. The product was used in a later reaction without further purification.

**3α-Acetoxy-20α-nitro-5β-pregnane (4).**—A solution of the 3α-acetoxy-20α-amine **2b** (3.0 g.) in chloroform (50 ml.) was added slowly (over 0.5 hr.) to a refluxing solution of *m*-chloroperbenzoic acid (12.0 g.) in chloroform (50 ml.). After a further 10 min. (total reaction time 40 min.), the reaction mixture was washed twice with 10% aqueous sodium sulfite, then twice with saturated potassium bicarbonate solution, and finally with water. The crude product obtained by removal of the chloroform *in vacuo* was dissolved in benzene and chromatographed on silica gel (90 g.). Elution with benzene–chloroform (3:7) gave the nitro compound **4** (2.15 g.). Crystallization from ether gave analytically pure **4**: m.p. 167–169°; $[\alpha]_D$ 0°; $\nu_{max}$ 1733, 1560, 1250 cm.$^{-1}$.

*Anal.* Calcd. for $C_{23}H_{37}NO_4$: C, 70.55; H, 9.53; N, 3.58. Found: C, 70.34; H, 9.40; N, 3.71.

The retention time on 1% QF-1 at 235°[24] was 28.7 min.; relative retention time ($\Delta^4$-cholesten-3-one = 1.0) was 1.12.

**Formation of the 20-Nitroso Dimer 11 from 3α-Acetoxy-20α-amino-5β-pregnane (2b).**—A solution of the 20α-amine **2b** (90 mg.) and *m*-chloroperbenzoic acid (137 mg.) in chloroform (10 ml.) containing finely powdered calcium carbonate (100 mg.) was stirred under reflux for 1 hr. The mixture was cooled and filtered, and the filtrate was washed successively with 10% aqueous sodium sulfite, 10% aqueous sodium bicarbonate, and water and evaporated *in vacuo*. The residue was crystallized from methylene chloride–petroleum ether to give the nitroso dimer **11** (14 mg.): m.p. 175–181°; $\lambda_{max}$ 298 mμ (ε 9000); $\nu_{max}$ 1753, 1250 (acetate), 1190 cm.$^{-1}$ (nitroso dimer).

*Anal.* Calcd. for $C_{46}H_{74}N_2O_6$: C, 73.56; H, 9.93; N, 3.73. Found: C, 73.78; H, 10.40; N, 3.74.

**3α-Acetoxy-20β-nitro-5β-pregnane (6).**—A solution of 3α-acetoxy-20β-amino-5β-pregnane (**5b**, 300 mg.) in chloroform (13 ml.) was added dropwise, over 15 min., to a refluxing solution of *m*-chloroperbenzoic acid (1.20 g.) in chloroform (10 ml.). After a further 25 min. (total reaction time 40 min.), the reaction mixture was worked up as in the other oxidations described above, and the crude product was chromatographed on silica gel (31 g.). Elution with benzene gave the 20β-nitro compound **6** (257 mg.). The analytical sample, obtained by crystallization from ether, showed m.p. 167–169°; $[\alpha]_D$ +43°; $\nu_{max}$ 1730, 1658, 1250 cm$^{-1}$.

*Anal.* Calcd. for $C_{23}H_{37}NO_4$: C, 70.55; H, 9.53; N, 3.58. Found: C, 70.49; H, 9.47; N, 3.71.

The retention time on 1% QF-1 at 235° was 25.0 min.; relative retention time ($\Delta^4$-cholesten-3-one = 1.0) was 0.97.

**3β-Nitro-5α-cholestane (8) and the Nitroso Dimer 12 from 3β-Amino-5α-cholestane (7).**—A solution of 3β-amino-5α-cholestane (**7**, 1.0 g.) in chloroform (15 ml.) was added dropwise, over 15 min., to a boiling solution of *m*-chloroperbenzoic acid (4 g.) in chloroform (15 ml.). After a further 10 min. (total reaction time, 25 min.), the solution was cooled and worked up exactly as described above for compounds **4** and **6**. The crude product was treated with boiling acetonitrile, and the hot solution was filtered quickly. The insoluble residue (271 mg.) proved to be the nitroso dimer **12**. Attempts to crystallize this dimer from ethyl acetate resulted in partial decomposition to

(24) Melting points were determined on the Kofler hot stage. Optical rotations were measured in chloroform solution, as were infrared spectra. Ultraviolet spectra were recorded using heptane solutions. Thin layer chromatography (t.l.c.) was carried out using 0.25-mm. layers of silica gel G or aluminum oxide G. The silica gel used for column chromatography was Davison Chemical Co. Grade 923. For gas–liquid partition chromatography, a Barber-Colman Series 5000 gas chromatograph was used, with argon ionization detector ($^{90}$Sr). The column was 1% QF-1 on 80–100 mesh Gas-Chrom P (which had been acid-washed and silanized with 5% dichlorodimethylsilane in toluene). The glass column tube was 8 ft. long, 5 mm. i.d. Operating conditions were column temperature, 235°; injector block, 280°; detector, 260°. Argon flow rate was 80 cc./min. at outlet (inlet pressure 18 p.s.i.). The *m*-chloroperbenzoic acid used in the oxidations was generously donated by FMC Corp. (New York) to whom our best thanks are due. This material was 85% pure.

(25) The solvent system, chloroform–methanol–ammonia, 132:12:0.9, was used.

(26) An authentic sample of this compound, kindly supplied by Dr. Nedelec, showed m.p. 202–206°, undepressed on admixture with our material, and showed the same $R_f$ value on thin layer chromatography. Our amine in methanol solution showed $[\alpha]_D$ +27°.

the 3-oxime, as shown by thin layer chromatography. However, chromatography of the partially decomposed dimer on silica gel and elution with benzene–petroleum ether (1:1) gave pure nitroso dimer **12**, homogeneous by t.l.c. (benzene–petroleum ether 2:1), m.p. 192–195°, $[\alpha]_D$ +16°, $\lambda_{max}^{heptane}$ 294 mμ (ε 8500), $\nu_{max}$ 1180 cm.$^{-1}$ (*trans*-nitroso dimer).

*Anal.* Calcd. for $C_{54}H_{94}N_2O_2$: C, 80.73; H, 11.80; N, 3.49. Found: C, 80.31; H, 11.43; N, 3.94.

The filtrate from the original treatment of the crude reaction product with boiling acetonitrile was then concentrated, when 3β-nitro-5α-cholestane (**8**, 482 mg.) crystallized out. This material showed double m.p. 99–101° and 109–112° (lit.[23] double m.p. 98–99° and 109–110°). The analytically pure **8** obtained by recrystallization from acetonitrile showed m.p. 111.5–112.5°, $[\alpha]_D$ +24° (lit.[23] $[\alpha]_D$ +23°).

**3α-Nitro-5α-cholestane (10) from 3α-Amino-5α-cholestane (9).**—A solution of 3α-amino-5α-cholestane (**9**, 1.0 g.) in chloroform (15 ml.) was added dropwise (over 35 min.) to a refluxing solution of *m*-chloroperbenzoic acid (5.0 g.) in chloroform (15 ml.). After a further 10 min. (total reaction time 45 min.), the reaction mixture was cooled and worked up as described for the preparation of compound **4**. The crude reaction product was chromatographed on silica gel (30 g.) when elution with benzene–petroleum ether (1:1) gave 3α-nitro-5α-cholestane (**10**, 300 mg.). Crystallization from acetone gave **10** as plates, m.p. 159–161°, $[\alpha]_D$ +29° (lit.[23] m.p. 157–160°, $[\alpha]_D$ +26°).

**Equilibration of the 20α- and 20β-Nitro Steroids 4 and 6. A. Sodium Hydrogen Carbonate in 95% Ethanol.**—The nitro steroid (**4** or **6**, 1.0 mg.) was dissolved in 1.0 ml. of a saturated solution of sodium hydrogen carbonate in 95% ethanol, and the solution was refluxed for 18 hr. The reaction mixture was taken to dryness under a stream of nitrogen, the residue was extracted with chloroform, and the chloroform was washed with water and evaporated to dryness under nitrogen. The bicarbonate treatment led to hydrolysis of the 3-acetoxy group as well as equilibration at C-20. In each case, therefore, the product was dissolved in pyridine (0.1 ml.) and acetic anhydride (0.02 ml.) and kept at 25° for 18 hr. The mixture was then evaporated to dryness under a jet of nitrogen and was dissolved in benzene (1 ml.) for gas chromatographic analysis. Separate experiments, conducted with the 3α-acetoxy-20-nitro compounds **4** and **6**, showed that the pyridine–acetic anhydride treatment caused no epimerization at C-20. The areas under the curves were measured by planimetry, and the relative proportions of the isomers were calculated from the results of a series of runs with synthetic mixtures containing known quantities of the pure isomers **4** and **6**. The results are accurate to ±5%.

**B. Tetramethylguanidine in Toluene.**—The nitro steroid (**2** or **4**, 2 mg.) was dissolved in toluene (2 ml.) containing tetramethylguanidine (0.2 ml.), and the solution was refluxed for 1 hr. The mixture was concentrated *in vacuo*, diluted with water, and extracted with chloroform. The chloroform extract was washed with water and evaporated to dryness *in vacuo*. The residue was then dissolved in benzene (2 ml.) for gas chromatographic analysis.

The areas under the curves were determined by planimetry, and the relative proportions of the compounds were calculated from the results of a series of synthetic mixtures of the two isomers. The results are summarized in Table II and are accurate to ±5%.

TABLE II
EQUILIBRIUM DATA FOR 20α- AND 20β-NITRO COMPOUNDS

| Conditions | α:β ratio — From 20α | From 20β |
|---|---|---|
| $NaHCO_3$–ethanol | 41:59 | 42:58 |
| Tetramethylguanidine–toluene | 43:57 | 39:61 |

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# New Enynolic Acids from *Acanthosyris*. Structures and Chemistry[1]

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The seed oil of *Acanthosyris spinescens* contains three previously unknown hydroxyacetylenic fatty acids. These are shown to be 7-hydroxy-*trans*-10,16-heptadecadien-8-ynoic acid (Ia), 9%; 8-hydroxy-*trans*-11,17-octadecadien-9-ynoic acid, 4%; and 7-hydroxy-*trans*-10-heptadecen-8-ynoic acid, 6%. The methyl ester Ib does not dehydrate readily in acidic methanol but instead undergoes slow etherification. The hydroxyl in the grouping –CH=CHC≡CCHOH– is less readily etherified than that in the isomeric grouping –C≡CCH=CHCHOH–. Two etherification products of Ib were detected: methyl 7-methoxy-*trans*-10,16-heptadecadien-8-ynoate and a dimethoxyallene that was not fully characterized. Two other derivatives of Ib have been synthesized: methyl 7-hydroxyheptadecanoate and *trans*-8-*trans*-10,16-heptadecatriene-1,7-diol.

In an earlier report[3] we discussed the isolation of a number of new acetylenic fatty acids from *Acanthosyris spinescens* (Mart. et Eich.) Griseb. (*Santalaceae*) seed oil. The nonoxygenated acid fraction included two unique acids having normal $C_{17}$ skeletons and a $C_{18}$ acid having an isolated triple bond. This present paper reports the isolation and structure proof of three new hydroxyacetylenic acids from *Acanthosyris* oil and describes some of the chemistry of the grouping –CH=CHC≡CCHOH–.[4]

The hydroxy acid fraction of *Acanthosyris* oil accounted for 20% of the total fatty acids.[3] Hydrogenation of a portion of the mixed hydroxy acids and gas–liquid partition chromatographic (glpc) analyses of the product indicated that both $C_{17}$ and $C_{18}$ hydroxy acids were present and that these occurred in a ratio of approximately 3:1.

The mixture of hydroxy acids was resolved by countercurrent distribution of the methyl esters. This separation was accomplished with a hexane–acetonitrile system in a Craig–Post[5] apparatus. The weight distribution obtained after 1500 transfers (Figure 1) showed separation of the esters into two peaks. The slower moving peak (tubes 50–110) was shown by its infrared spectrum (strong band at 10.95 μ) to contain esters having terminal double bonds (Ib and IIb, Chart I). The faster moving peak contained

(1) Presented in part before the American Oil Chemists' Society, Houston, Texas, April 25–28, 1965.

(2) One of the laboratories of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(3) R. G. Powell and C. R. Smith, Jr., *Biochemistry*, in press.

(4) The structure of Ia was indicated in a preliminary communication: R. G. Powell and C. R. Smith, Jr., *Chem. Ind.* (London), 470 (1965).

(5) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.