The Oxidation of Steroid 9(11)-Olefins in Nitrosyl Fluoride Solutions[1]

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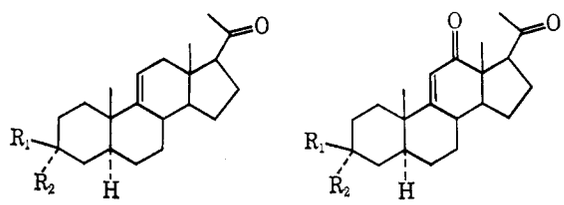
Steroid 9(11)-olefins, when reacted at 50° in a solution of nitrosyl fluoride in ethyl acetate, form the corresponding α,β-unsaturated 12-ketones. The structure of the products was proven by partial synthesis from a known sapogenin. The reaction is not due to the presence of the impurity nitrogen dioxide, since under the same conditions this reagent forms 17-nitro derivatives.

Although nitrosyl fluoride (NOF), the most reactive halide of nitrous acid, has been known for over 60 years,[2] its use as a reagent in organic chemistry has been limited to relatively few types of compounds. In the older literature the only report of its reaction with an organic substance is that of treatment of NOF with benzene under unspecified conditions. The product, nitrosobenzene, was identified only by odor and color.[3] More recently, a number of investigators have reported the reaction of NOF with fluoroolefins,[4] fluoro ketones,[5] and Δ^5-steroids.[6] These reactions were carried out either with neat reagents or in the presence of nonpolar solvents such as carbon tetrachloride or freons. In all the cases reported, the reactions could be explained as involving an initial addition of elements of NOF to the carbon–carbon or carbon–oxygen double bond. Either the addition products or derivatives resulting from hydrolysis or further reaction with NOF were isolated.

We wish to report that the reaction of NOF with some relatively hindered 9(11)-steroid olefins in a polar medium gives rise to a different type of reaction.

When nitrosyl fluoride was added to a solution of 3β-hydroxy-5α-pregn-9(11)-en-20-one acetate (**1a**) in ethyl acetate and the reaction was maintained at 50° for 18 hr, the starting material was completely consumed and was transformed into an unstable intermediate which was not characterized. When the crude reaction product was chromatographed on neutral alumina, a 20% yield of a new product was obtained which analyzed for $C_{23}H_{32}O_4$ and showed a uv maximum at 238 mμ (ϵ 12,700). The nmr and ir spectra were consistent with the formulation of the product as the diketone derived from the oxidation of the starting material at position 12. This compound, 3β-hydroxy-5α-pregn-9(11)-ene-12,20-dione acetate (**2a**), had not been reported in the literature; however, the closely related 3-ketone, 5α-pregn-9(11)-ene-3,12-20-trione (**2b**), had been previously characterized.[7]

The nitrosyl fluoride reaction was therefore carried out on the corresponding ketone (**1b**) and a product was obtained in 30% yield which was suspected to be compound **2b**. Comparison of the infrared spectrum of



1 **2**

a, R_1 = OAc; R_2 = H
b, R_1; R_2 = =O
c, R_1; R_2 = O, 5β-H

our oxidation product with that of an authentic sample[8] showed them to be similar but not identical. We therefore felt that this contradiction could best be clarified by an independent synthesis of ketone **2b**. This was accomplished according to Scheme I. Hecogenin acetate (**3**) was converted into 9(11)-dehydrohecogenin acetate (**4**) by oxidation with selenium dioxide.[9] The product was then degraded by a modification of the standard method[10] to 3β-hydroxy-5α-pregna-9(11),16-diene-12,20-dione acetate (**5**). This bis-α,β-unsaturated ketone possessed certain unusual properties which will be mentioned later. When subjected to catalytic hydrogenation in the presence of pyridine and ethyl acetate until 1 mol of hydrogen was taken up, the dienedione was converted into its 16,17-dihydro derivative **6**. Saponification and oxidation with chromium trioxide–acetone gave authentic compound **2b**. This sample was identical in all respects with our nitrosyl fluoride oxidation product but different from the "authentic sample."

This apparent anomaly was clarified when 5β-pregn-9(11)-ene-3,20-dione (**1c**) was allowed to react with nitrosyl fluoride in ethyl acetate. 12-Ketone **2c** was formed which was in fact identical with the sample

(1) This work was supported by contract with the Schering Corp., Bloomfield, N. J.

(2) O. Ruff and K. Stäuber, *Z. Anorg. Chem.*, **47**, 190 (1905).

(3) O. Ruff, W. Meuzel, and W. Neumann, *Z. Anorg. Allg. Chem.*, **208**, 293 (1932).

(4) I. L. Knunyants, E. G. Bykhovskaya, V. N. Frosin, and Ya. M. Kisel, *Dokl. Akad. Nauk SSSR*, **132**, 123 (1960), *Chem. Abstr.*, **54**, 20840*i* (1960); D. A. Barr and R. N. Haszeldine, *J. Chem. Soc.*, 1151 (1960); S. Andreades, *J. Org. Chem.*, **27**, 4163 (1962).

(5) S. Andreades, *ibid.*, **27**, 4157 (1962).

(6) G. A. Boswell, Jr., *ibid.*, **31**, 991 (1966); G. A. Boswell, Jr., *Chem. Ind.* (London), 1929 (1965).

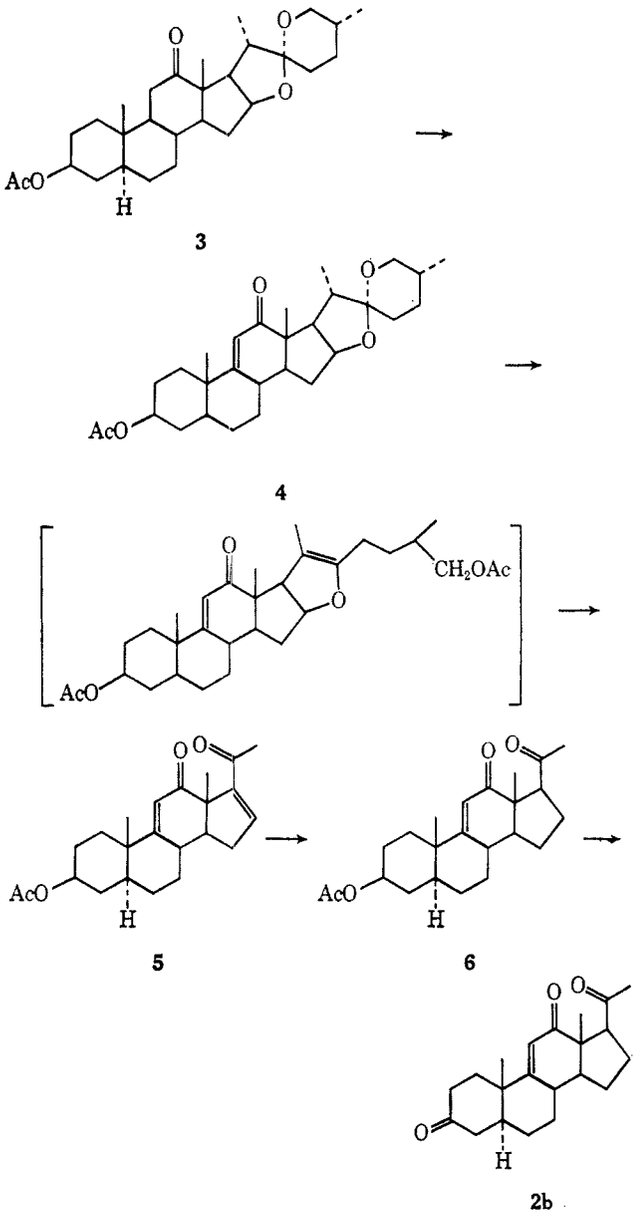
(7) R. N. Jones, P. Humphries, and K. Dobriner, *J. Amer. Chem. Soc.*, **72**, 956 (1950).

(8) We wish to thank Dr. T. F. Gallagher, custodian of the late Dr. Dobriner's collection, for his help in the characterization of these compounds.

(9) A. Bowers, E. Denot, M. B. Sanchez, F. Neumann, and C. Djerassi, *J. Chem. Soc.*, 1859 (1961).

(10) M. E. Wall and S. Serota, *Tetrahedron*, **10**, 238 (1960).

SCHEME I



procured from Dr. Gallagher, and which had been mistakenly reported to be the 5α compound.[11]

The hydrogenation of either dienedione **5** or ketone **6** under ordinary conditions in the absence of pyridine gave a 3:2 mixture of the saturated dione acetate and the previously reported[12] 12β-hydroxy-3β-acetoxy-pregnan-20-one. Since the saturated dione acetate was not reduced at all under these conditions, the reduction of the 12 carbonyl group in the unsaturated ketone must be especially enhanced by the presence of the 9(11) double bond, presumably due to complexing with the catalyst. Therefore, although the rates of hydrogenation of the 16 and the 9(11) double bonds are significantly different as expected, the existence of a third competing site precludes the possibility of a cleanly selective hydrogenation of the 16 double bond under ordinary conditions. However, the addition of pyridine to the reaction mixture completely inhibited the reduction of the 12 carbonyl group and further enhanced the difference between the rates of hydrogenation of the 16 double bond and the hindered 9(11) double bond, so that ketone **6** was readily obtained from dienedione **5**.

The ultraviolet spectrum of dienedione **5** is unusual, showing λ_{max} at 229 mμ (ε 17,000). This is in contrast to other $\Delta^{9(11)}$-12 ketones and Δ^{16}-20 ketones which normally absorb at 237 and 240 mμ, respectively. The hypsochromic shift caused by a 12 carbonyl group on the Δ^{16}-20 ketone system has been observed previously[13] and has been explained by assuming that the Δ^{16}-20 ketone exists in a *s-trans* orientation which causes the two carbonyl oxygen atoms attached to C-12 and C-20 to approach one another very closely. The resulting dipole–dipole interaction increases the energy required to excite the chromophore resulting in a hypsochromic shift. The effect evidently does not involve twisting about the 17–20 single bond, since the other conjugated ketone at C-12 also undergoes a hypsochromic shift, and in this case twisting about the single bond between the double bond and the carbonyl group is severely restricted because of ring strain.

The dienedione is also unusual in its extreme reactivity toward nucleophiles,[14] reacting with methoxide ion in methanol even faster at C-16 than 3β-hydroxy-16-pregnene-12,20-diene acetate.

The exact nature of the oxidizing species which is present when NOF is dissolved in ethyl acetate at room temperature and reacted with a steroid at 50° is as yet unknown. It was suggested by a referee that NOF is not present at all in the solution but is transformed into oxides of nitrogen which are the true oxidizing species. To clarify this point several additional experiments were undertaken.

First we attempted to investigate the constitution of the nitrosyl fluoride employed.[15] The mass spectrum of the gaseous reagent obtained directly from the cylinder showed the presence of silicon tetrafluoride, nitric oxide and hydrogen fluoride, normal reaction products of NOF with the glass inlet line of the mass spectrometer. In addition, it was possible to detect the presence of some nitrogen dioxide present as an impurity in the reagent. Nitrogen dioxide adds to unhindered olefins to form primarily nitrite esters of nitro alcohols.[16] In the case of a hindered steroid olefin, however, we were not aware that this reaction had been previously attempted. We therefore treated steroid **1a** in ethyl acetate with nitrogen dioxide to see if this was the agent responsible for the formation of the conjugated ketone (Scheme II).

(11) The 5β ketone has been synthesized earlier by another route [P. Hegner and T. Reichstein, *Helv. Chim. Acta*, **26**, 721 (1943)] and its properties are in agreement with our preparation.

(12) W. J. Adams, D. N. Kirk, D. K. Patel, V. Petrow, and I. A. Stuart-Webb, *J. Chem. Soc.*, 870 (1955).

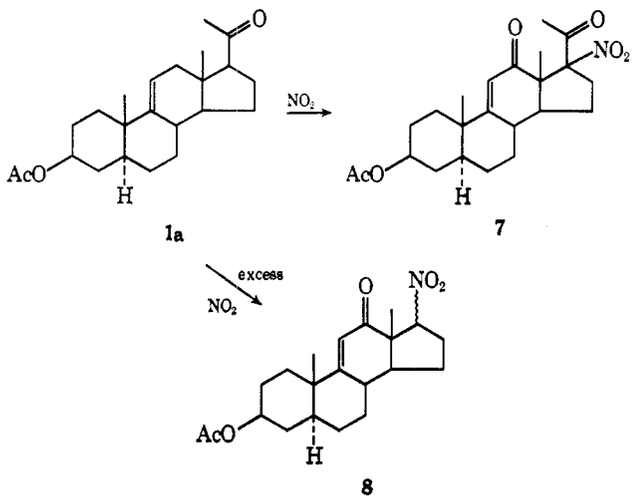
(13) G. P. Mueller, R. E. Stobaugh, and R. S. Winniford, *J. Amer. Chem. Soc.*, **75**, 4888 (1953).

(14) G. S. Abernethy and M. E. Wall, *J. Org. Chem.*, **34**, in press.

(15) The nitrosyl fluoride used for all of the experiments described in this paper was the commercial product purchased from the Ozark-Mahoning Co., Tulsa, Okla. 74119.

(16) H. Shechter, *Rec. Chem. Progr.*, **25**, 55 (1964).

SCHEME II



In the presence of excess NO_2 the reaction mixture was very complex. However a small amount of a crystalline product was obtained whose structure can be inferred from the structure of the starting material and from spectral data to be compound **8**. In the presence of a very limited quantity of nitrogen dioxide, the nitro ketone **7** was obtained along with substantial amounts of starting material. In neither reaction mixture was the presence of any of the product **2a** detectable.

We therefore conclude that the conversion of compounds **1** to **2** is brought about by the combined presence of the reagent nitrosyl fluoride in the solvent ethyl acetate. This behavior can be likened to the reaction of hindered olefins with other oxidizing agents to form conjugated ketones.[17]

Experimental Section[18]

3β-Hydroxy-5α-pregn-9(11)-ene-12,20-dione Acetate (2a).—3β-Hydroxy-5α-pregn-9(11)-en-20-one acetate[19] (1.0 g, 2.79 mmol) was dissolved in 40 ml of ethyl acetate in an all-polyethylene system. Nitrogen was bubbled through the reaction mixture which was cooled in an ice bath. Nitrosyl fluoride was then bubbled into the reaction mixture which on saturation turned a brilliant green-blue. The solution was transferred under nitrogen pressure into a monel flask, which was then sealed and maintained at about 50° overnight. The reaction mixture was washed with water and saturated sodium chloride solution and the aqueous layers were back-washed with ethyl acetate. The combined organic solutions were dried over sodium sulfate and evaporated to give 1.26 g of a chartreuse oil. The oil was dissolved in a minimum amount of 1:1 benzene–hexane and chromatographed on 120 g of alumina. The column was eluted with 1500 ml of 1:1 benzene–hexane, and 750 ml of 1% methanol in 1:1 benzene–hexane in a three-flask gradient. Compound **2a** (206 mg, 2070), obtained from the chromatography in crystalline form, was recrystallized from methylene chloride–hexane and melted at 138–139°: ir (KBr) λ_{max} 1735, 1704, 1678, 1809, 1248 cm^{-1}; nmr C_{11} τ 4.28 (d, J = 2), C_{17} 6.80 (t, J = 8), C_{21} 7.60, -OAc, 7.98, C_{19} 8.89, C_{18} 9.16; uv ϵ_{238}^{max} 12,660.

Anal. Calcd for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.41; H, 8.82, and no F or N.

5α-Pregn-9(11)-ene-3,12,20-trione (2b).—5α-Pregn-9(11)-ene-3,20-dione (0.913 g, 2.9 mmol) in 170–180 ml of ethyl acetate, was treated as in the previous reaction to yield 1.14 g of a crude gum. The gum was absorbed from chloroform onto 20 g of alumina which was then added atop a column of 125 g of alumina as a slurry in 1:1 benzene–hexane. The column was eluted with 1800 ml of 1:1 benzene–hexane, and 900 ml of 2% methanol in 1:1 hexane–benzene in a three-flask gradient system. From the column was obtained 290 mg of crude product (30%) which, after florisil cleanup and recrystallization, melted at 246.5–248.5°: ir (KBr) λ_{max} 1708, 1670, 1600 cm^{-1}; nmr C_{11} τ 4.23 (d, J = 2), C_{17} 6.78 (t, J = 8), C_{21} 7.60, C_{19} 8.69, C_{18} 913; uv ϵ_{236}^{max} 12,800.

Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.75; H, 8.43.

5β-Pregn-9(11)-ene-3,12,20-trione (2c).—5β-Pregn-9(11)-ene-3,20-dione (500 mg, 1.59 mmol) in 50 cc of ethyl acetate was allowed to react as in the previous example to give 750 mg of a crude oil. The oil was chromatographed on 5 g of alumina and added, in a slurry with 50% benzene–hexane, to a 13:1 column of 100 g of alumina. The column was eluted with 1000 cc of 50% benzene–hexane and 500 cc of 2% methanol in 50% benzene–hexane in a three-flask gradient. The 160 mg which was eluted was rechromatographed on a 20 × 40 cm × 1 mm plate of silica gel HF, developing with 1:3 acetone–carbon tetrachloride. The major band was fluorescent and yielded 43 mg of yellow crystals upon elution with chloroform. Florisil cleanup, sublimation and recrystallization from methylene chloride–hexane afforded 17.8 mg, mp 181.5–183°. The infrared spectrum was identical with that of an authentic sample: ir (KBr) λ_{max} 1705, 1668, 1600 cm^{-1}; nmr C_{11} τ 4.04 (d, J = 2.5), C_{17} 6.76 (t, J = 8), C_{21} 7.57, C_{19} 8.70, C_{18} 9.12; uv ϵ_{237}^{max} 12,800.

9(11)-Dehydrohecogenin acetate was prepared by the selenium dioxide oxidation of hecogenin acetate as described by Bowers, *et al.*[9] Its nmr spectrum showed the characteristic vinyl proton absorption at 342 cps.

3β-Hydroxy-5α-pregna-9(11),16-diene-16,20-dione Acetate (5).—9(11)-Dehydrohecogenin acetate (10.2 g) was refluxed in 20 ml of acetic anhydride containing 10 ml of pyridine and 3.4 g of methylammonium chloride for 4 hr. The reaction mixture was cooled and poured into ice water. The mixture was extracted with two portions of ethyl acetate. The combined extracts were washed with water and saturated sodium chloride solution, then dried over sodium sulfate and evaporated to give 14 g of an oil.

The oil was dissolved in 20 ml of 1,2-dichloroethane and 20 ml of glacial acetic acid and the mixture cooled to −15° in a Dry Ice–acetone bath. A solution of 2.6 g of chromium trioxide in 20 ml of 90% acetic acid was added over a 15-min period. The reaction mixture was stirred at −5° for 45 min. Ethanol (10 ml) was added and the reaction mixture refluxed for 5 hr. The mixture was washed twice with water and once with saturated sodium chloride solution. The aqueous washes were backwashed with four portions of methylene chloride. The combined organic extracts were dried over magnesium sulfate, filtered and evaporated to give 11.6 g of a black oil. Chromatography on 1 kg of silica gel, eluting with 4 l. each of 5, 10 and 25% acetone in carbon tetrachloride, provided 4.1 g of crude product. This was passed through a cleanup column of *ca.* 10 g of alumina in ether and recrystallized from acetone–hexane to give 2.85 g of compound **5** melting at 150–154°, containing no more than 5% of its 16-dehydro derivative by nmr and mass spectral estimation: ir (mull) λ_{max} 3075, 1737, 1696, 1680, 1602, 1250 cm^{-1}; nmr C_{16} τ 3.33 (m), C_{11} 4.32 (d, J = 2), C_2 7.63, -OAc 7.95, C_{19} 8.73, C_{18} 8.85; uv ϵ_{229}^{max} 17,000.

Anal. Calcd for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.56; H, 8.12.

3β-Hydroxy-5α-pregn-9(11)-ene-12,20-dione Acetate (6).—To

(17) H. R. Bentley, J. A. Henry, D. S. Irvine, and F. S. Spring, *J. Chem. Soc.*, 3673 (1953).

(18) Unless otherwise indicated, chromatography was carried out on Woelm neutral, activity III alumina. Ultraviolet spectra were determined in absolute ethanol. Nmr spectra were determined in deuteriochloroform at 60 Mc, with a tetramethylsilane internal standard. Unless otherwise indicated, all nmr peaks were singlets; J values are in cycles per second.

(19) The $\Delta^{9,11}$ steroids used in this study were kindly given to us by Dr. H. Herzog, Schering Corp., Bloomfield, N.J.

30 ml of ethyl acetate was added 3 ml of pyridine and 60 mg of 5% palladium on charcoal catalyst. The mixture was hydrogenated to saturation, then 300 mg of compound **5** in 30 ml of ethyl acetate was added. The approximate rate of hydrogen uptake was 0.12 mequiv/min for the first mole, abruptly decreasing to 0.012 mequiv/min. Approximately half of the reaction mixture was removed after the uptake of 1 mequiv. It was filtered and evaporated to give the expected product contaminated with less than 5% of the 9(11) dihydro derivative (by nmr estimation) and no discernible 12β-hydroxypregnane. One crystallization from methanol afforded material melting at 134–137°.

The hydrogenation of the remaining half of the reaction mixture was permitted to proceed to completion. Filtration and evaporation gave 3β-hydroxy-5α-pregnane-12,20-dione acetate uncontaminated by any other steroid, by nmr analysis.

Treatment of 3β-Hydroxy-5α-pregn-9(11)-en-20-one Acetate (1a) with Excess Nitrogen Dioxide in Ethyl Acetate at 50°.—The steroid (1.08 g) was dissolved in 40 ml of dry ethyl acetate in a monel flask and the solution chilled in an ice bath. Gaseous nitrogen dioxide was passed into the reaction mixture for 1 hr. The reaction vessel was sealed and incubated at 53° for 18 hr. The reaction mixture was concentrated under vacuum. Ethyl acetate was added to the residue. The solution was washed with water and saturated sodium chloride solution. Both aqueous washes were extracted with a portion of ethyl acetate. The combined organic solutions were dried over sodium sulfate and evaporated to give a brown oil which appeared to be a complex mixture containing no starting material by tlc. The oil was adsorbed onto 10 g of alumina and placed atop a column of 110 g of alumina in 1:1 benzene–hexane. The column was eluted with a three-flask gradient of 1800 ml of 1:1 benzene–hexane and 900 ml of 2% methanol in 1:1 benzene–hexane, then with 1000 ml of 2% methanol in 1:1 benzene–hexane. Only one compound (60 mg) was obtained in crystalline form. Upon recrystallization from methylene chloride–hexane, it melted at 202–206° with profuse sweating. Nmr, ir and mass spectra confirmed the structure to be 3β-hydroxy-17ξ-nitroandrost-9(11)-en-12-one acetate **8**: ir (mull) 3062, 1730, 1670, 1595, 1545, 1306, 1245, 1158, 1032, 783 cm^{-1}; nmr ($CDCl_3$) C_{11} τ 4.22, C_{17} 4.96 (t, J = 9), C_3 5.32 (m), –OAc 7.97, C_{19} 8.89, C_{18} 8.95; uv ϵ_{237}^{max} 14,600; mass spectrum, 375.2062 (calcd for $C_{21}HN_{29}O_5$, 375.2046).

Reaction of 3β-Hydroxy-5α-pregn-9(11)-en-20-one Acetate (1a) with a Limited Amount of Nitrogen Dioxide.—In 60 ml of dry ethyl acetate was dissolved 0.58 g of nitrogen dioxide. The solution was added to the steroid (1.0 g) in a monel flask. The vessel was sealed and incubated at 50° for 18 hr. The reaction mixture was washed with saturated sodium bicarbonate solution and saturated sodium chloride solution, then dried over sodium sulfate and evaporated to give 0.95 g of brown oil which, by tlc, contained mostly starting material in combination with many other products. The oil was dissolved in 1:1 ethyl acetate–benzene and passed through 120 g of alumina. A yellow oil (550 mg) was eluted, which consisted of at least seven components by tlc with two spots predominating. This oil was chromatographed on 75 g of silica gel, eluting with 3 l. of 2–3% acetone in carbon tetrachloride. Eluted first was 290 mg of a yellow oil estimated to be 80% starting material by its nmr spectrum and by tlc. About 50 mg of a crystalline product was then obtained, which upon recrystallization from methylene chloride–hexane formed colorless needles of 3β-hydroxy-17ξ-nitro-5α-pregn-9(11)-ene-12,20-dione acetate (**7**): mp 199–210°; ir (mull) 3062, 1745, 1722, 1680, 1602, 1550, 1278, 1252, 1145, 1030 cm^{-1}; nmr C_{11} τ 4.12, C_{21} 7.44, –OAc 7.96, C_{19} 8.90, C_{18} 9.19; uv ϵ_{237}^{max} 11,500; mass spectrum, 417.2172 (calcd for $C_{23}H_{31}NO_6$, 417.2151).

Registry No.—**2a**, 18266-99-4; **2b**, 18267-00-0; **2c**, 18267-01-1; **5**, 18267-02-2; **7**, 18267-03-3; **8**, 18267-04-4; nitrosyl fluoride, 7,789-25-5.

The Alkaloids of *Peschiera lundii* (D.C.) Miers.[1] Isolation and Structure Elucidation of Voacristine Pseudoindoxyl and Iboxygaine Hydroxyindolenine

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Voacristine pseudoindoxyl ($C_{22}H_{28}N_2O_5$) and iboxygaine hydroxyindolenine ($C_{20}H_{26}N_2O_3$) were isolated from *Peschiera lundii* (D.C.) Miers. Their structures were determined on the basis of spectral data and confirmed by partial synthesis from related alkaloids. In addition, voacangine, coronaridine, voacristine, 20-epivoacristine, iboxygaine, ibogaine, olivacine, and vobasine were found in the same plant. Most of the iboga alkaloids isolated were oxidized to the corresponding hydroxyindolenines and rearranged to pseudoindoxyls, several of which were chemically characterized for the first time.

As part of a continuing study of the chemotaxonomy and biological activities of selected portions of the *Apocynaceae*,[2] we became interested in a Brazilian representative of the *Tabernaemontaneae*, identified as *Peschiera lundii* (D. C.) Miers.[3] The close botanical relationship between *Peschiera* and *Tabernaemontana* species has often led to confusion and either name has been assigned to a species, according to a botanist's individual preference. The detailed isolation and characterization of several alkaloids from *P. lundii* is now reported. In the course of determining the structure of two new alkaloids from this plant, and because of the recent isolation[4,5] of a number of hydroxyindolenines and pseudoindoxyls of iboga alkaloids, several known

(1) Problems in Chemotaxonomy. V.

(2) For example, see J. A. Weisbach, R. F. Raffauf, O. Ribeiro, E. Macko, and B. Douglas, *J. Pharm. Sci.*, **52**, 350 (1963); M. P. Cava, S. S. Tjoa, Q. A. Ahmed, and A. I. daRocha, *J. Org. Chem.*, **33**, 1055 (1968).

(3) The plant material used in this study was collected by Dr. Aparicio Duarte near Porto Seguro in the state of Bahia, Brazil. His assistance in the collection and identification of the material is gratefully acknowledged. A voucher specimen, no. 6828, has been deposited in the Herbarium Bradeanum, Rio de Janeiro, Brazil.

(4) C. Hootele, R. Levy, M. Kaisin, J. Pecher, and R. H. Martin, *Bull. Soc. Chim. Belges*, **76**, 300 (1967).

(5) B. C. Das, E. Fellion, and M. Plat, *C. R. Acad. Sci. Paris*, **C264**, 1765 (1967).