

## TURKISH TOBACCO—II

### ISOLATION AND CHARACTERIZATION OF 12 $\alpha$ -HYDROXY-13-EPIMANOYL OXIDE

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(Received 1 May 1961; in revised form 10 August 1961)

**Abstract**—A new unsaturated diterpene alcohol has been isolated from Turkish tobacco. Characterization studies have shown that the alcohol is 12 $\alpha$ -hydroxy-13-epimanoyl oxide. In connection with these studies, conclusive chemical evidence has been obtained confirming the previously assigned stereochemistry of manoyl oxide and its 13-epimer.

THE present paper describes further study of the hexane extract of one thousand pounds of Turkish tobacco.<sup>1</sup> Continuing the fractionation of the extract by chromatography and countercurrent partitions has led to the isolation of small quantities of a new diterpene alcohol, C<sub>20</sub>H<sub>34</sub>O<sub>2</sub>, m.p. 141–142°C, to which the structure 12 $\alpha$ -hydroxy-13-epimanoyl oxide (I) is assigned.

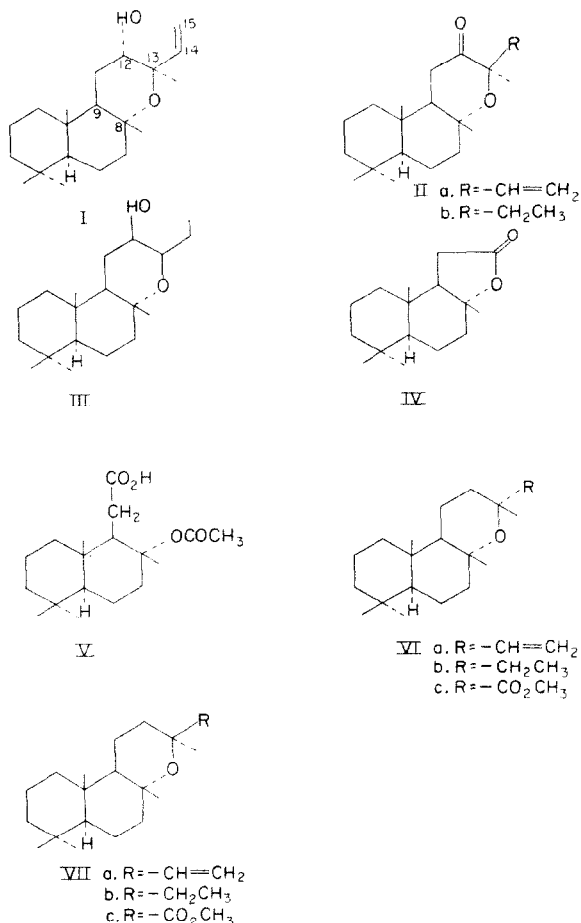
The infrared spectrum (Fig. 1) of 12 $\alpha$ -hydroxy-13-epimanoyl oxide demonstrated the presence of olefinic (6.17  $\mu$ ) and hydroxyl (3.00, 9.33  $\mu$ ) groupings, and indicated that an ether grouping (9.18  $\mu$ ) might also be present. Absorption in the fingerprint region at 10.09, 10.80 and 10.89  $\mu$  suggested a CH<sub>2</sub>=CHR grouping. The NMR spectrum (Fig. 2) showed that the compound contained five methyl groups having  $\tau$  values of 9.25, 9.21, 9.14, 8.80 and 8.76 p.p.m. referred to tetramethylsilane. A single terminal vinyl group was demonstrated by the characteristic quadruplets centered at 3.88, 5.01, and 5.17 p.p.m., the latter two of which were not fully resolved. The absence of further splitting of the quadruplet at 3.88 p.p.m. indicated that the vinyl grouping is attached to a carbon bearing no protons. The peak at 8.08 p.p.m. is assigned to the hydroxyl proton.

The mass spectrum showed that 12 $\alpha$ -hydroxy-13-epimanoyl oxide had a molecular weight of 306. Quantitative hydrogenation of the alcohol gave equivalent weights of 297 and 313, indicating the presence of one double bond. Active hydrogen analysis showed that the alcohol is monohydric. Since the infrared spectrum lacked carbonyl absorption, the second oxygen atom must be in an ether grouping. The possibility of epoxide or hemiacetal oxygen was eliminated by failure of 12 $\alpha$ -hydroxy-13-epimanoyl oxide to react with lithium aluminum hydride in refluxing tetrahydrofuran.

Oxidation of 12 $\alpha$ -hydroxy-13-epimanoyl oxide or its dihydro derivative with chromic anhydride-pyridine gave the respective ketones (IIa, IIb) in good yield. The mass spectral fracture patterns of the ketones gave evidence that the functional groups were located at C-8 and C-12 through C-16<sup>2</sup>. Comparison of the fracture patterns of the ketones and 12 $\alpha$ -hydroxy-13-epimanoyl oxide with those of related known com-

<sup>1</sup> J. A. Giles and J. N. Schumacher, *Tetrahedron* **14**, 246 (1961).

<sup>2</sup> A. H. Laurene and G. W. Young, paper in preparation.



pounds confirmed<sup>2</sup> their assignment to the group of diterpenes typified by sclareol,<sup>3,4</sup> manool,<sup>5</sup> and manoyl oxide.<sup>5-7</sup>

Stereospecific reduction of dihydro-12-keto-13-epimanoyl oxide (IIb) with lithium tri-*t*-butoxyaluminumhydride<sup>8</sup> gave the equatorial alcohol, dihydro-12 $\beta$ -hydroxy-13-epimanoyl oxide (III). This alcohol differed from the one obtained by hydrogenation of the natural product, and was oxidizable back to the starting ketone (IIb). Therefore, the hydroxyl group in the tobacco component must be in the axial, or  $\alpha$ -, configuration. The epimeric alcohols also exhibited infrared absorption at 994 cm<sup>-1</sup> (axial) and 1005 cm<sup>-1</sup> (equatorial) in agreement with their assigned configurations.<sup>9</sup> Examination of the NMR spectrum of (I) revealed a triplet ( $J = 3.5$  cps) at 5.92 p.p.m. which was assigned to the proton attached to the carbon bearing the hydroxyl, and a doublet ( $J = 3.5$  cps) at 8.25 p.p.m. assigned to two protons attached to an adjacent carbon.

<sup>3</sup> J. A. Barltrop, D. B. Bigley and N. A. J. Rogers, *Chem. & Ind.* 400 (1959).

<sup>4</sup> L. Ruzicka, C. F. Seidel and L. L. Engel, *Helv. Chim. Acta* 25, 621 (1942).

<sup>5</sup> J. R. Hosking, *Ber. Dtsch. Chem. Ges.* 69, 780 (1936).

<sup>6</sup> R. Hodges and R. I. Reed, *Tetrahedron* 10, 71 (1960).

<sup>7</sup> J. R. Hosking and C. W. Brandt, *Ber. Dtsch. Chem. Ges.* 68, 37 (1935).

<sup>8</sup> O. H. Wheeler and J. L. Mateos, *Chem. & Ind.* 395 (1957).

<sup>9</sup> D. H. R. Barton, *J. Chem. Soc.* 1027 (1953).

Since this  $J$  value corresponds to axial–equatorial and equatorial–equatorial spin–spin interactions,<sup>10</sup> it follows that the proton on the hydroxyl-bearing carbon is in the equatorial configuration, confirming assignment of the hydroxyl group to the axial configuration.

Ozonization of 12 $\alpha$ -hydroxy-13-epimanoyl oxide (I) followed by oxidative hydrolysis of the ozonide gave the lactone (IV)<sup>4</sup> and the acetoxy acid (V).<sup>4</sup> The lactone (IV) provided a fragment of known constitution containing sixteen of the twenty carbon atoms. The acetoxy acid (V) gave a known fragment of eighteen carbons and indicated that C-16 was in a methyl group. A second ozonization followed by reductive hydrolysis of the ozonide gave formaldehyde as expected from the vinyl grouping. Therefore, nineteen of the carbon atoms appear in the degradation products. The remaining carbon was undoubtedly split off by secondary reaction of the ozonide with ozone, probably as carbon dioxide. Because of the secondary degradation, it is reasonable to conclude that one or both of the carbonyl groups in the acetoxy acid must have been formed after cleavage of the double bond, and that they do not necessarily indicate the functions of these carbons prior to ozonolysis. It is safe to assume, however, that both of these carbons were originally attached to a functional group, either hydroxyl, ether, or double bond.

12-Keto-13-epimanoyl oxide (IIa) was subjected to a Wolff–Kishner reduction.<sup>11</sup> The olefinic double bond was reduced<sup>12</sup> along with the ketone function to give the known diterpene, dihydro-13-epimanoyl oxide<sup>13</sup> (VIIb). Because 12 $\alpha$ -hydroxy-13-epimanoyl oxide contains a vinyl grouping and since the functional groups are restricted to the terminal five carbon atoms, it follows that the hydroxyl group is attached to C-12.

Considering the physical evidence and the degradation products, it had been anticipated that Wolff–Kishner reduction of the ketone IIa would produce a manoyl oxide. The accompanying reduction of the double bond by hydrazine<sup>12</sup> then indicated that the product was dihydromanoyl oxide or its 13-epimer. However, the physical constants were not in close agreement with those reported for dihydromanoyl oxide<sup>14</sup> (VIb) or for dihydro-13-epimanoyl oxide<sup>13</sup> (VIIb). It was, therefore, necessary to obtain these compounds for comparison. Treatment of sclareol with acetic anhydride by Ohloff's method<sup>13</sup> gave the epimanoyl oxide (VIIa), m.p. 100.5–101.5°,  $[\alpha]_D^{25} + 35.3^\circ$ , having an infrared spectrum identical to that reported.<sup>13</sup> Hydrogenation of the epimanoyl oxide then provided the dihydro oxide VIIb, m.p. 31°,  $[\alpha]_D^{25} + 21.9^\circ$ , identical with the Wolff–Kishner reduction product.

First examination indicates that Wolff–Kishner reduction of the ketone IIa has led to an abnormal product. This is not necessarily the case since hydrazine is known to hydrogenate a variety of compounds.<sup>12,15</sup> In the present work, it was shown that 12 $\alpha$ -hydroxy-13-epimanoyl oxide is quantitatively reduced to its dihydro derivative with hydrazine in refluxing ethanol with no inversion of configuration. In a second Wolff–Kishner reduction of the ketone IIa, the crude hydrazone was isolated prior to reduction. Following reduction, two products, the dihydro oxide VIIb and an azine, were

<sup>10</sup> J. A. Pople, W. G. Schneider and H. J. Bernstein, *High-resolution Nuclear Magnetic Resonance* pp. 394–396. McGraw-Hill, New York (1959).

<sup>11</sup> Huang-Minlon, *J. Amer. Chem. Soc.* **68**, 2487 (1946).

<sup>12</sup> F. Aylward and M. Sawistowska, *Chem. & Ind.* 404 (1961), and references cited therein.

<sup>13</sup> G. Ohloff, *Liebigs Ann.* **617**, 134 (1958).

<sup>14</sup> J. R. Hosking and C. W. Brandt, *Ber. Dtsch. Chem. Ges.* **67**, 1173 (1934).

<sup>15</sup> C. R. Scholfield, E. P. Jones, J. Nowakoska, E. Selke and H. J. Dutton, *J. Amer. Oil Chem. Soc.* **38**, 208 (1961).

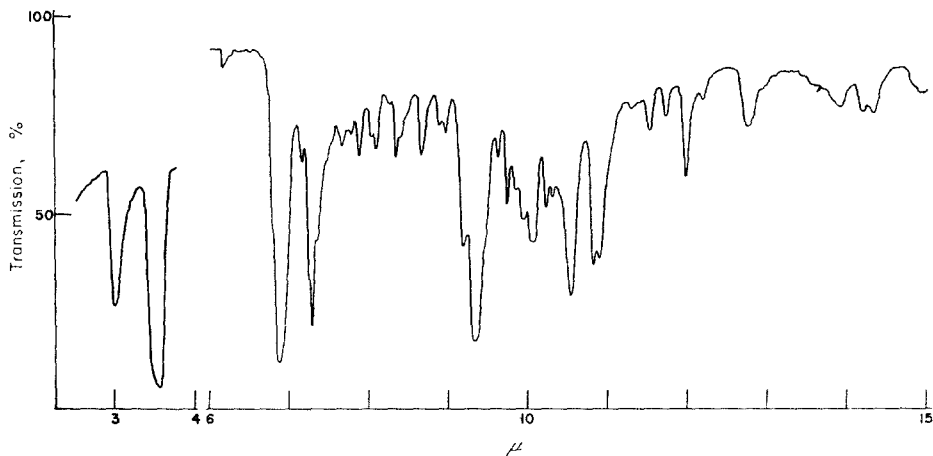


FIG. 1. Infrared spectrum of 12 $\alpha$ -hydroxy-13-epimanoyl oxide in Nujol mull. Perkin-Elmer Model 21.

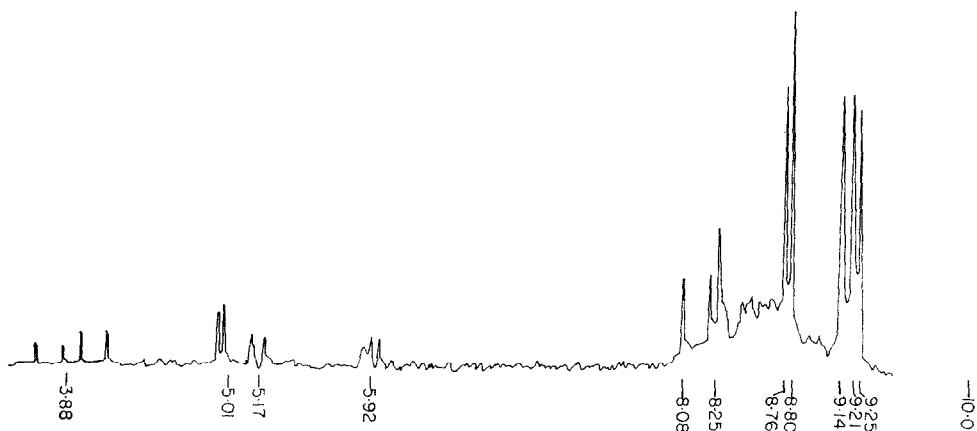


FIG. 2. 12 $\alpha$ -Hydroxy-13-epimanoyl oxide. NMR spectra in  $\text{CDCl}_3$  at 60 mc in p.p.m. relative to tetramethyl silane as the internal standard (10 p.p.m.). Varian Model V-4300B.

isolated. The azine was hydrolyzed with difficulty to give the dihydro ketone IIb. Thus it is apparent that the double bond was reduced during formation of the hydrazone and prior to the Wolff-Kishner reduction. However, this does not obviate the possible opening of the oxide ring during reduction of the hydrazone. That some ring opening occurred is inferred by the low yield (30 per cent) of dihydro-13-epimanoyl oxide. Opening of the oxide ring followed by reclosure, however, probably would affect the stereochemistry at C-13. This sequence should be expected to involve racemization rather than inversion at C-13. The fact that dihydro-13-epimanoyl oxide was accompanied by none of its epimer then indicates that reclosure of the oxide ring does not occur and that the reduction product is normal. Further support of the assigned C-13 configuration of 12 $\alpha$ -hydroxy-13-epimanoyl oxide is suggested by the close similarity of its NMR spectrum (Fig. 2) with that of 13-epimanoyl oxide (Fig. 3). The differences in the spectra are those which can be attributed to the 12 $\alpha$ -hydroxyl grouping. It

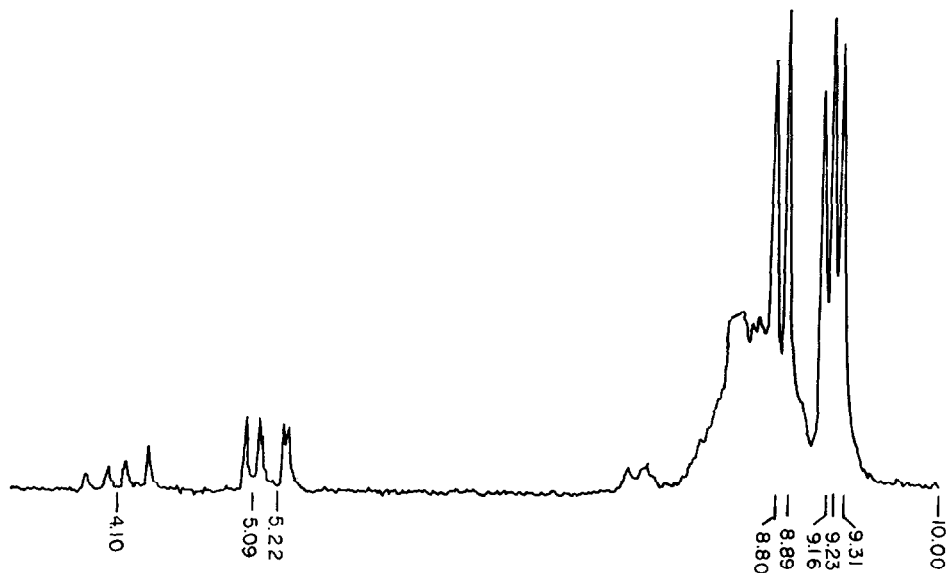


FIG. 3. 13-Epimanoyl oxide.

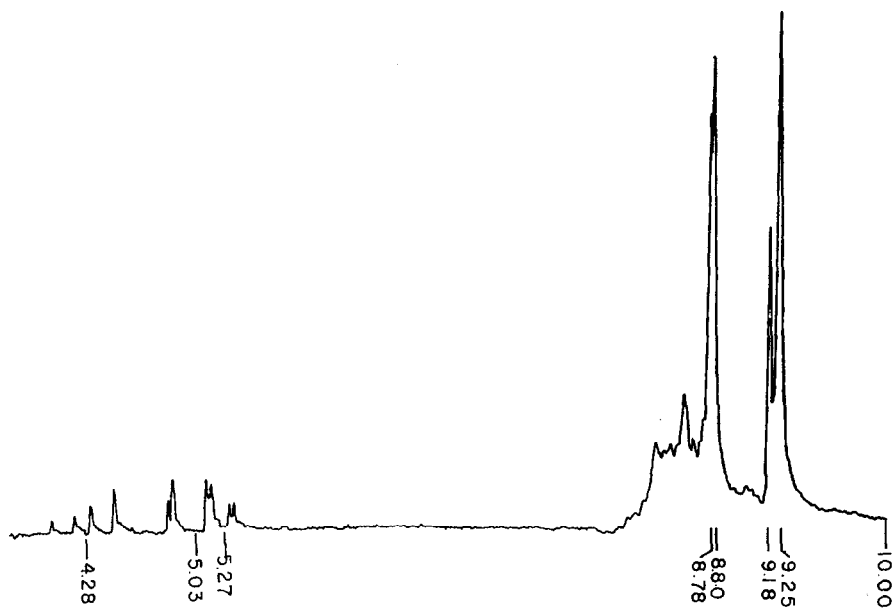


FIG. 4. Manoyl oxide.

should also be noted that the similarity between the spectra of I and manoyl oxide (Fig. 4) is not nearly as great.

Ohloff<sup>13</sup> suggested that either natural manoyl oxide was impure or that his synthetic oxide was epimeric at C-8 or C-9. Hodges and Reed<sup>6</sup> subsequently showed that the synthetic oxide was 13-epimanoyl oxide. Furthermore, on the basis of mass spectrometric fracture patterns, Hodges and Reed<sup>6</sup> assigned the 16-methyl group of

manoyl oxide to the  $\beta$ -configuration and proposed the structure VIa for the oxide. Since this evidence did not seem sufficiently conclusive, confirmatory experiments were undertaken. Manoyl oxide and 13-epimanoyl oxide were transformed into the corresponding methyl manoylates (VIc, VIId), and the saponification rates of these esters were determined. Methyl manoylate was found to react thirty-eight times as fast as methyl 13-epimanoylate. This shows that the carbomethoxy group of methyl manoylate has the less hindered  $\alpha$ -configuration and confirms the configurational assignment based on the mass spectra.

### EXPERIMENTAL

All melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Elemental analyses were performed by the Huffman Microanalytical Laboratories, Wheatridge, Colorado. Active hydrogen analyses were performed in this laboratory by tritium exchange.<sup>16</sup>

*Isolation of 12 $\alpha$ -hydroxy-13-epimanoyl oxide.* Fraction 2<sup>1</sup> (142 g), after standing for two months at  $-15^\circ$ , deposited solids which were removed by filtration. The filtrate (126 g) was divided into six equal portions. Each portion was partitioned between twelve 100 ml hexane layers and twelve 100 ml 9:1 methanol-water layers. Concentration of hexane layers 0-3 gave 45.6 g of hydrocarbons. The remaining layers were combined to give 74 g of syrup which partially solidified after several weeks at  $-15^\circ$ . After filtering, the syrup (62 g) was partitioned between twenty-five 100 ml pentane layers and twenty-five 100 ml 17:3 methanol-water layers giving Fraction 1,<sup>17</sup> 3.1 g; Fraction 2, 9.2 g; Fraction 3, 15.1 g; Fraction 4, 26.6 g; Fraction 5, 5.7 g; and Fraction 6, 1.8 g.

After standing for three months at  $-15^\circ$ , Fraction 3 partly crystallized. Filtration of the mixture gave 800 mg of crystals. Recrystallization from hexane gave 12 $\alpha$ -hydroxy-13-epimanoyl oxide (I), m.p. 141-142°,  $[\alpha]_D^{25} +44^\circ$  (CHCl<sub>3</sub>). (Found: C, 78.34; H, 11.27; Act. H, 0.35; Mol. Wt. 306 (mass spectrometer). C<sub>20</sub>H<sub>34</sub>O<sub>2</sub> requires: C, 78.37; H, 11.18; Act. H, 0.33%; Mol. Wt., 306). The mother liquor was combined with Fraction 4. Further partitioning followed by chromatography of selected fractions on silicic acid gave an additional 900 mg of 12 $\alpha$ -hydroxy-13-epimanoyl oxide.

*Dihydro-12 $\alpha$ -hydroxy-13-epimanoyl oxide.* Hydrogenation of 111 mg of 12 $\alpha$ -hydroxy-13-epimanoyl oxide in ethanol over platinum required 8.42 ml of hydrogen at 26° and 746 mm of Hg giving a value of 1.03 double bond. A second experiment gave a value of 0.98 double bond. Chromatography of the reduced material on alumina with 99:1 chloroform-ethanol gave 102 mg of dihydro-12 $\alpha$ -hydroxy-13-epimanoyl oxide, m.p. 142-143°,  $[\alpha]_D^{25} +30^\circ$  (CHCl<sub>3</sub>),  $\nu_{\max}$  994 cm<sup>-1</sup>. (Found: C, 77.88; H, 12.06. C<sub>20</sub>H<sub>36</sub>O<sub>2</sub> requires: C, 77.86; H, 11.76%.)

A solution of 95 mg of 12 $\alpha$ -hydroxy-13-epimanoyl oxide in 20 ml of 10% ethanolic hydrazine hydrate was refluxed for 7 hr, then cooled and allowed to stand overnight. The solution was poured into 100 ml of water and extracted with 100 ml of hexane. After washing with water, the hexane layer was dried with sodium sulfate and concentrated to give 94 mg of dihydro-12 $\alpha$ -hydroxy-13-epimanoyl oxide, m.p. 141.5-142°, identical with the catalytic hydrogenation product.

*12-Keto-13-epimanoyl oxide (IIa).* Solid 12 $\alpha$ -hydroxy-13-epimanoyl oxide (100 mg) was added to a solution of 500 mg of chromic anhydride in 5 ml of dry pyridine at 25°, then stirred for 3 hr. After being allowed to stand overnight, the mixture was poured into water and extracted with ether. The extract was concentrated and chromatographed on silicic acid with 3:1 benzene-hexane to give 73 mg (74% yield) of 12-keto-13-epimanoyl oxide, m.p. 92-93° (Found: C, 78.89; H, 10.54; Mol. Wt., 304 (mass spectrometer). C<sub>20</sub>H<sub>32</sub>O<sub>2</sub> requires: C, 78.89; H, 10.60%; Mol. Wt., 304).

*Dihydro-12-keto-13-epimanoyl oxide (IIb).* Oxidation of dihydro-12 $\alpha$ -hydroxy-13-epimanoyl oxide as described above gave 105 mg of crude product. The product was recovered unchanged after shaking for 2 hr with 3 ml of Tollens reagent at 60°. Chromatography on silicic acid with benzene gave 80 mg of dihydro-12-keto-13-epimanoyl oxide, m.p. 101° (Found: C, 78.30; H, 11.11; Mol. Wt., 306 (mass spectrometer). C<sub>20</sub>H<sub>34</sub>O<sub>2</sub> requires: C, 78.37; H, 11.18%; Mol. Wt., 306).

*Oxidation of 12 $\alpha$ -hydroxy-13-epimanoyl oxide ozonide. Isolation of the acid (V) and lactone (IV).* Ozonized oxygen was bubbled through a solution of 200 mg of 12 $\alpha$ -hydroxy-13-epimanoyl oxide in 50 ml of chloroform for 15 min at  $-10^\circ$ . The ozonide obtained after evaporation of the solvent was

<sup>16</sup> J. A. Giles, *Analyt. Chem.* **32**, 1716 (1960).

<sup>17</sup> The similarity of infrared spectra is used as the criterion for grouping fractions.

oxidized with 75 ml of 3% hydrogen peroxide.<sup>18</sup> Following decomposition of the excess peroxide with palladized charcoal, the mixture was extracted with ether. The extract was separated into 31 mg of acidic product and 111 mg of neutral material.

Recrystallization of the acidic product from hexane gave the acetoxy acid (V), m.p. 155–157°. Ruzicka *et al.*<sup>4</sup> report m.p. 159°. The infrared spectrum was identical with that of an authentic specimen.

Chromatography of the neutral product on silicic acid with 9:1 benzene–ether gave 65 mg of the lactone (IV), identified by its infrared spectrum.

*Reduction of 12 $\alpha$ -hydroxy-13-epimanoyl oxide ozonide. Isolation of formaldehyde.* Following ozonization of 299 mg of 12 $\alpha$ -hydroxy-13-epimanoyl oxide, the ozonide was refluxed for one hour with zinc dust in 1% acetic acid, then steam distilled. The distillate was collected in 125 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. Extraction of the distillate mixture with chloroform followed by chromatography on silicic acid with 19:1 hexane–ether gave 90 mg of formaldehyde 2,4-dihydrophenylhydrazone, m.p. and mixture m.p., 164–165°. The infrared spectrum was identical with that of an authentic sample.

*Wolff–Kishner reduction of 12-keto-13-epimanoyl oxide.* Run I—A mixture of 147 mg of 12-keto-13-epimanoyl oxide, 2 ml of 85% hydrazine hydrate, and 11 ml of diethylene glycol was stirred and gently refluxed at 110–125° for 3 hr. The reflux condenser was removed and the temperature was slowly raised to 200°. Two pellets of potassium hydroxide were added. The reflux condenser was replaced; stirring and heating were continued for 15 hr at 225–230°. After cooling, the mixture was poured into 100 ml of water and extracted twice with benzene. Chromatography of the extract on alumina with 4:1 hexane–benzene gave 38 mg of dihydro-13-epimanoyl oxide. The product was combined with 25 mg of oxide from Run II. Further chromatography on Florisil with 7:1 hexane–benzene gave 43 mg of dihydro-13-epimanoyl oxide (VIIb), m.p. 26–28°,  $[\alpha]_D^{25} + 21.1^\circ$  (CHCl<sub>3</sub>). The infrared spectrum was identical with that of authentic dihydro-13-epimanoyl oxide.

Run II—A solution of 319 mg of 12-keto-13-epimanoyl oxide and 3 ml of 100% hydrazine hydrate in 10 ml of acetic acid was heated for 4 hr at 100°, then cooled and poured into water. Extraction with ether–hexane then gave 377 mg of the crude hydrazone. The crude hydrazone and 0.5 g of potassium hydroxide were dissolved in 10 ml of diethylene glycol, then stirred and heated for 8 hr at 200°. After cooling, the mixture was poured into 100 ml of water and extracted with hexane. Chromatography of the extract on alumina with 5:1 hexane–benzene gave 25 mg of dihydro-13-epimanoyl oxide. Further elution with 1:1 benzene–hexane gave 113 mg of a *ketazine*, m.p. 180–181°. (Found: C, 79.13; H, 11.08; N, 4.65; M.W. 520 (Rast). C<sub>40</sub>H<sub>68</sub>N<sub>2</sub>O<sub>2</sub> requires: C, 78.89; H, 11.25; N, 4.60; M.W. 609.)

A solution of 90 mg of the azine and 5 ml of acetic acid in 20 ml of acetone was refluxed for 66 hr, then cooled and concentrated. Chromatography of the residue on silicic acid with 3:1 benzene–hexane gave 15 mg of dihydro-12-keto-13-epimanoyl oxide, identified by its infrared spectrum.

*Dihydro-12 $\beta$ -hydroxy-13-epimanoyl oxide* (III). A solution of 206 mg of dihydro-12-keto-13-epimanoyl oxide in 20 ml of ether was added slowly to 1.2 g of lithium tri-*t*-butoxyaluminumhydride in 50 ml of refluxing ether.<sup>8</sup> The mixture was stirred and refluxed for 2 hr, then decomposed with ethanol and 3 N potassium hydroxide. The organic layer was concentrated, then chromatographed on alumina and on silicic acid to give 176 mg of *dihydro-12 $\beta$ -hydroxy-13-epimanoyl oxide*, m.p. 140–141°,  $[\alpha]_D^{25} + 38^\circ$  (CHCl<sub>3</sub>)  $\nu_{\max}$  1005 cm<sup>-1</sup> (Found: C, 77.91; H, 11.71. C<sub>20</sub>H<sub>36</sub>O<sub>2</sub> requires: C, 77.86; H, 11.76%). Oxidation of dihydro-12 $\beta$ -hydroxy-13-epimanoyl oxide with chromic anhydride in pyridine gave dihydro-12-keto-13-epimanoyl oxide.

*13-Epimanoyl oxide.* Sclareol (20 g) treated with acetic anhydride according to Ohloff<sup>13</sup> gave 0.6 g of 13-epimanoyl oxide following chromatography on alumina. Recrystallization from acetone and from methanol gave crystals, m.p. 100.5–101.5°,  $[\alpha]_D^{25} + 35.3^\circ$  (CHCl<sub>3</sub>). Ohloff<sup>13</sup> reports m.p. 94–96°,  $[\alpha]_D^{25} + 37.5^\circ$ . The infrared spectrum was identical with that reported by Ohloff.<sup>13</sup>

*Dihydro-13-epimanoyl oxide.* Hydrogenation of 90 mg of 13-epimanoyl oxide was accomplished in ethanol over platinum at 25° and atmospheric pressure. Chromatography of the product on Florisil with 9:1 hexane–benzene gave 77 mg of dihydro-13-epimanoyl oxide, m.p. 31°,  $[\alpha]_D^{25} + 21.9^\circ$  (CHCl<sub>3</sub>) (Found C, 82.42; H, 12.44. Calc. for C<sub>20</sub>H<sub>36</sub>O: C, 82.12; H, 12.40%). Ohloff<sup>13</sup> reports  $[\alpha]_D^{20} + 17^\circ$  (CHCl<sub>3</sub>).

<sup>18</sup> C. S. Marvel, W. M. Schilling, D. J. Shields, C. Bluestein, O. R. Irvin, P. G. Sheth and J. Honig, *J. Org. Chem.* **16**, 838 (1951).

*Methyl manoylate (VIc).* Oxidation of 10.3 g of manoyl oxide according to Hosking and Brandt<sup>7</sup> gave 1.84 g of crude manoylic acid. The acid was esterified with diazomethane. Chromatography of the ester on silicic acid with 3:1 benzene-hexane, followed by recrystallization from aqueous methanol gave 0.98 g of methyl manoylate, m.p. 84–85°,  $[\alpha]_D^{25} + 9^\circ$  (CHCl<sub>3</sub>). Hodges and Reed<sup>6</sup> report m.p. 83–85°,  $[\alpha]_D + 14^\circ$ .

*13-Epimanoylic acid.* Oxidation<sup>7</sup> of 20.9 g of 13-epimanoyl oxide gave 10.1 g of crude 13-epimanoylic acid. Recrystallization from hexane and from aqueous methanol gave *13-epimanoylic acid*, m.p. 152–154°,  $[\alpha]_D^{25} + 49.9^\circ$  (CHCl<sub>3</sub>),  $\nu_{\max}$  1768 cm<sup>-1</sup> (CCl<sub>4</sub>) (Found: C, 74.53; H, 10.29; Mol. Wt., 308 (mass spectrometer). C<sub>19</sub>H<sub>32</sub>O<sub>3</sub> requires: C, 73.98; H, 10.46%; Mol. Wt., 308).

*Methyl 13-epimanoylate (VIc).* 13-Epimanoylic acid (13 g) was treated with diazomethane to give 13 g of crude methyl ester. Two recrystallizations from aqueous methanol gave *methyl 13-epimanoylate*, m.p. 99–100°,  $[\alpha]_D^{25} + 30.5^\circ$  (CHCl<sub>3</sub>) (Found: C, 74.50; H, 10.42. C<sub>20</sub>H<sub>34</sub>O<sub>3</sub> requires: C, 74.49; H, 10.63%).

*Rates of saponification of the epimeric methyl manoylates.* Partial saponification of 100 mg of ester was carried out in 10.00 ml of 0.26 N ethanolic potassium hydroxide at 58°. The solution was chilled, acidified with 27.00 ml of 0.1 N ethanolic p-toluenesulfonic acid, and titrated to the phenol red end point with 0.04432 N potassium hydroxide. A blank determination was made with each sample. The first order rate constants were calculated: methyl manoylate,  $125 \times 10^{-3} \text{ min}^{-1}$ ; methyl 13-epimanoylate,  $3.32 \times 10^{-3} \text{ min}^{-1}$ .

*Acknowledgements*—The authors wish to thank Messrs. Paul H. Ayers, Earl Hester, T. C. James, H. G. Hilliard, and R. L. Montgomery for technical assistance. We are indebted to J. J. Whalen and J. L. Stewart for the infrared spectra, to George W. Young for the mass spectra and to L. E. Lewallen for the NMR spectra. We are grateful to Drs. E. E. van Tamelen, W. G. Brown, Murray Senkus, A. H. Laurene, L. R. Haefele, P. H. Latimer, and C. E. Teague for many helpful discussions in the course of this work.