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TURKISH TOBACCO—I

ISOLATION AND CHARACTERIZATION OF α- AND β-LEVANTENOLIDE

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Abstract—Two hitherto unknown diterpene lactones have been isolated from Turkish tobacco. Characterization studies show that these compounds, now called α- and β-levantenolides, are represented by I and II. The levantenolides differ only in being epimeric at C_{12}, and are closely related to labdanolic acid.

FRACTIONATION of the hexane extract of Turkish tobacco has yielded two lactones, α- and β-levantenolide,[1] belonging to the group of diterpenes typified by sclareol and labdanolic acid.

The infrared spectra (Figs. 1 and 2) of α-levantenolide (I) and β-levantenolide (II), $C_{20}H_{30}O_3$, were very similar, suggesting that the compounds were closely related. Absorption at 12·1 μ indicated the presence of the grouping $R_2C{=}CHR$. The carbonyl absorption at 5·75 μ suggested that both compounds are α,β-unsaturated γ-lactones.[2] The absence of hydroxyl or other carbonyl absorption required that the remaining oxygen be in an ether grouping. The NMR spectra[3] of the lactones were almost identical, showing one vinyl proton and five methyl groups, one of which is attached to a doubly-bound carbon beta to a carbonyl grouping. The partial structure,

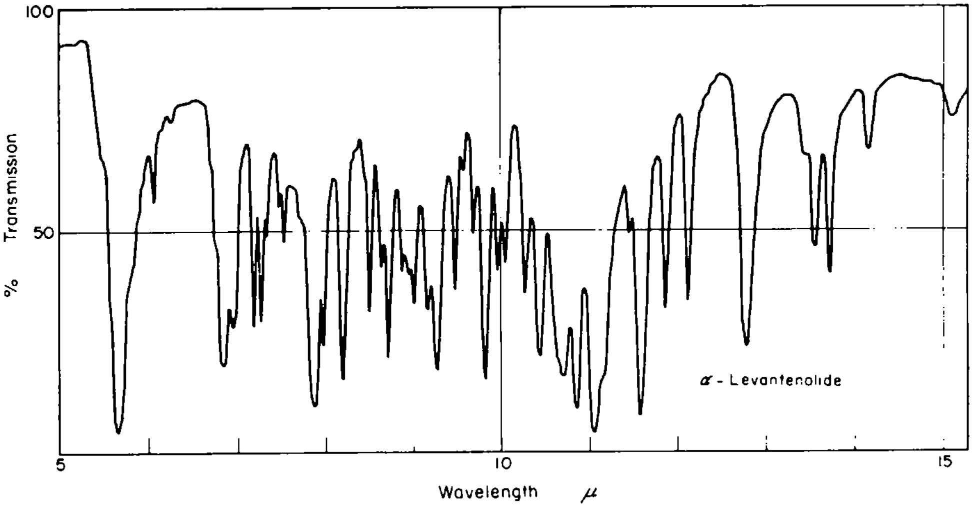

FIG. 1.

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[1] The name levantenolide was chosen to denote the functional groups as well as the fact that these lactones are found in tobacco commonly grown in the Near East.

[2] J. F. Grove and H. A. Willis, *J. Chem. Soc.* 877 (1951).

[3] Varian Associates, Palo Alto, California.

RC(CH_3)=CHC=O, then was apparent. The mass spectrum of α-levantenolide showed a parent mass of 318. Quantitative hydrogenation of the lactones gave equivalent weight values of 315, 309 and 277, indicating the presence of only one double bond.

The close relationship of α- and β-levantenolides was shown in several ways. Reduction of either lactone with lithium aluminum hydride gave the same triol, m.p. 145–146°. Reaction of either lactone with hydrazine provided the same pyridazone derivative (III), m.p. 212–213°. Saponification of either lactone followed by lactonization of the resultant acid gave predominantly α-levantenolide. Thus, any reaction which involves opening of the lactone rings will lead to a common product. *A priori*, α- and β-levantenolides can differ only in the configurations of the carbon atoms to which the lactone rings are attached. This facile interchange can occur only if the third oxygen atom is also attached to the carbon which changes configuration; i.e. α- and β-levantenolides must be the lactones of a hemi-ketal or a hemi-acetal. This conclusion is supported by the formation of a pyridazone upon reaction of the lactones with hydrazine.

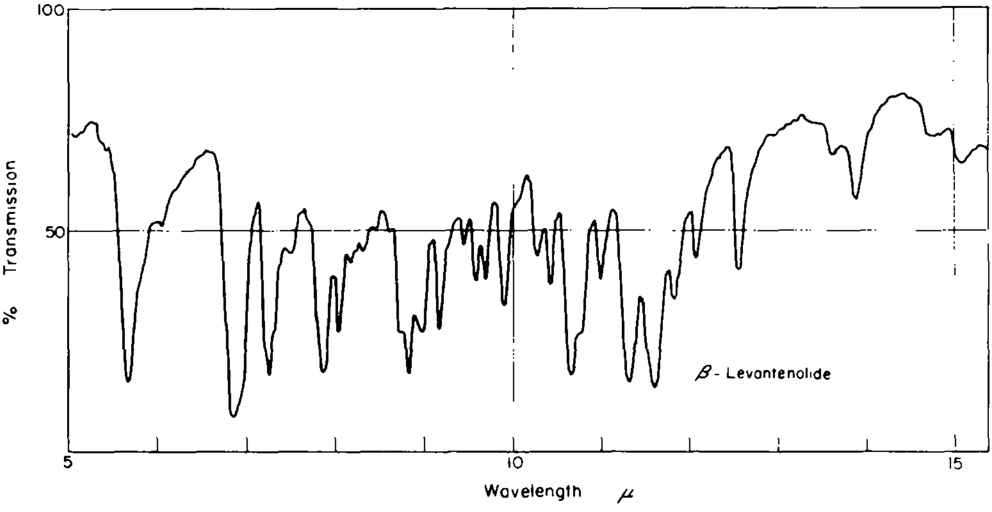

Fig. 2.

Ozonization of α-levantenolide followed by oxidative hydrolysis of the ozonide with aqueous hydrogen peroxide led to the formation of the lactone (IV) and oxalic acid. The degradation to the lactone (IV)[4] provided a fragment of known constitution containing sixteen of the twenty carbon atoms. Oxalic acid provided the terminal carbon atoms which must include the lactone carbonyl group and one of the doubly-bound carbon atoms. The ozonolysis product has, therefore, undergone secondary cleavage. This is explained when it is understood that the ozonolysis product is an α-diketone which in turn is readily cleaved with hydrogen peroxide[5] or with ozone.

Ozonization of α-levantenolide was repeated. The ozonide was reduced with lithium aluminum hydride to give the diol (V), m.p. 132°, and the triol (VI), m.p.

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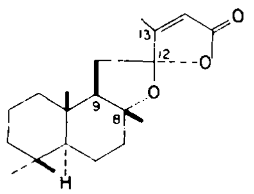
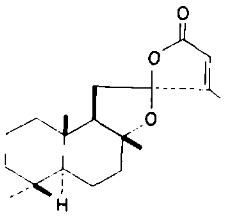
[4] M. Stoll and M. Hinder, *Helv. Chim. Acta* **36**, 1984 (1953); M. Hinder and M. Stoll, *Ibid.* **36**, 1995 (1953).

[5] R. C. Fuson, *Advanced Organic Chemistry* p. 236. John Wiley, New York (1950).



I



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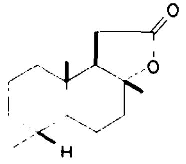
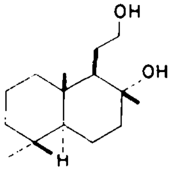
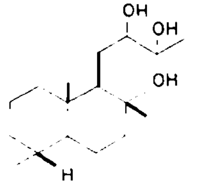


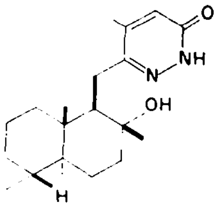
IV



V



VI



III

159·5–160·5°. Oxidation of the triol (VI) with lead tetraacetate gave acetaldehyde, isolated as the 2,4-dinitrophenylhydrazone in 75 per cent yield.

The saponification experiments have demonstrated that α-levantenolide is more stable than β-levantenolide, and indeed, it occurs to a greater extent in tobacco leaf. Construction of models shows that the formation of the lactone rings is sterically influenced by the methyl groups attached to carbons 8 and 13. This influence deters the 13-methyl group from assuming the position required for ring closure to give the lactone (II). Therefore, the structures I and II are proposed for α- and β-levantenolides respectively.

β-Levantenolide was converted via the dihydrolactone to the dihydropyridazone derivative. Treatment of the dihydropyridazone with potassium hydroxide under Wolff–Kishner conditions,[6] followed by esterification of the acidic products, gave methyl labdanolate,[7,8] m.p. 72–73°, and methyl 13-epilabdanolate,[7] m.p. 71–72°. Thus, α- and β-levantenolides are diterpenes of the class typified by sclareol,[9] manoöl,[10] labdanolic acid[7,8] and cativic acid.[11]

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[6] Huang-Minlon, *J. Amer. Chem. Soc.* **68**, 2487 (1946).

[7] S. Bory and E. Lederer, *Croat. Chem. Acta* **29**, 157 (1958).

[8] J. D. Cocker and T. G. Halsall, *J. Chem. Soc.* 4262 (1956).

[9] L. Ruzicka, C. F. Seidel and L. L. Engel, *Helv. Chim. Acta* **25**, 621 (1942); L. Ruzicka and M. M. Janot, *Ibid.* **14**, 645 (1931); J. A. Barltrop, D. B. Bigley and N. A. J. Rogers, *Chem. & Ind.* 400 (1959).

[10] J. R. Hosking and C. W. Brandt, *Ber. Dtsch. Chem. Ges.* **68**, 1311 (1935); J. R. Hosking, *Ibid.* **69**, 780 (1936).

[11] H. H. Zeiss and F. W. Grant, Jr., *J. Amer. Chem. Soc.* **79**, 1201 (1957).

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.[12]

Extraction of Turkish tobacco

One thousand pounds of aged, sun-cured Turkish tobacco, grown in Yugoslavia, were ground in a hammer mill to pass a ¼ in. screen. The tobacco was agitated and refluxed for 2 hr with 900 gallons of hexane, then 428 gallons of extract were siphoned off. Hexane (200 gallons) was added, then the mixture was agitated and refluxed for 0·5 hr and 436 gallons of extract were siphoned off. Hexane (445 gallons) was added, the mixture was agitated, and 645 gallons of extract were removed by water displacement. The total hexane extract was concentrated to 110 gallons, which contained 5·8 kg of solids, and extracted three times with 120 gallons of 9:1 methanol–water. Concentration of the combined aqueous methanol layers gave 2·8 kg of extract.

Fractionation of the extract

The aqueous methanol extract was chromatographed in 200 g portions using 5:1 Magnesol-Celite in a column 250 mm × 150 mm (dia.) and collecting the eluate, 15 l. of 1:1 hexane–ether, until a yellow band was about to be eluted. Concentration of the eluate from fourteen such columns gave 882 g of material which was fractionated by a second chromatogram using the same adsorbent. In chromatogram 2, the eluting solvents varied from 17:3 to 4:1 hexane–ether providing a separation into the following fractions:[13] Fraction 1, 158 g; Fraction 2, 142 g; Fraction 3, 185 g; and Fraction 4, 104 g.

On standing, Fraction 3 partially crystallized and 8·35 g of α-levantenolide (I) was removed by filtration. The filtrate was chromatographed on 5:1 Magnesol-Celite using hexane–ether mixtures of graded polarity. Appropriate fractions were partitioned between hexane and 90% methanol. Further partitioning of selected fractions using pentane and 4:1 methanol–water allowed separation of 0·73 g of β-levantenolide (II) and a further 0·3 g of α-levantenolide.

Crude β-levantenolide (4·7 g) crystallized from Fraction 4. The mother liquors were partitioned successively with hexane–90% methanol, hexane–70% methanol and pentane–70% methanol, and selected fractions were chromatographed on silicic acid. In this manner, an additional 1·3 g of β-levantenolide was obtained.

α-Levantenolide (I)

Recrystallization of the crude tobacco lactone from hexane gave α-levantenolide, m.p. 210°, $[\alpha]_D^{25}$ +60·4° ($CHCl_3$) (Found: C, 75·19; H, 9·32; Mol. wt. 318 (mass spectrometer). $C_{20}H_{30}O_3$ requires: C, 75·43; H, 9·58%; Mol. wt. 318).

Hydrogenation of α-levantenolide

Hydrogenation of 79·4 mg of α-levantenolide in ethanol over 30% Pd-C required 8·2 ml of hydrogen at 25° and 741 mm of Hg giving a value of 1·15 double bond. A second experiment gave a value of 1·03 double bond. Filtration of the mixture gave 85 mg of crude product. Recrystallization from hexane gave *α-levantanolide*, m.p. 144–146° (Found: C, 74·72; H, 10·13; Mol. wt. 320 (mass spectrometer). $C_{20}H_{32}O_3$ requires: C, 74·96; H, 10·01%; Mol. wt. 320).

β-Levantenolide (II)

The crude lactone isolated from Turkish tobacco was crystallized from hexane giving *β-levantenolide*, m.p. 208–209°, $[\alpha]_D^{25}$ −59·6° ($CHCl_3$) (Found: C, 75·33; H, 9·58. $C_{20}H_{30}O_3$ requires: C, 75·43; H, 9·58%).

Hydrogenation of β-levantenolide

Hydrogenation of 103 mg of β-levantenolide was accomplished over 30% Pd-C in ethanol with 8·2 ml of hydrogen at 24° and 736 mm of Hg giving a value of 1·01 double bond. Filtration and

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[12] J. A. Giles, *Analyt. Chem.* **32,** 1716 (1960).

[13] The similarity of infrared spectra is used throughout the isolation as the criterion for grouping fractions.

concentration of the mixture gave 126 mg of crude product. Recrystallization from ethanol–hexane gave *β-levantanolide*, m.p. 192–194° (Found: C, 74·27; H, 10·02. $C_{20}H_{32}O_3$ requires: C, 74·96; H, 10·01%).

Reduction of α-levantenolide with lithium aluminum hydride

Reduction of 50 mg of α-levantenolide was accomplished quantitatively according to Nystrom and Brown[14]. Recrystallization of the crude product from hexane-ethyl acetate gave *levanten*-8α, 12,15-*triol*, m.p. 145–146° (Found: C, 73·77; H, 11·43; Act. H, 0·97. $C_{20}H_{36}O_3$ requires: C, 74·02; H, 11·18; Act. H, 0·93%). Levanten-8α, 12,15-triol was also obtained by the similar reduction of β-levantenolide.

Reduction of α-levantanolide

Quantitative reduction of 83 mg of α-levantanolide according to Nystrom and Brown[14] gave *levantan*-8α, 12,15-*triol*, m.p. 121–122·5°, after recrystallization from hexane (Found: C, 73·14; H, 11·88. $C_{20}H_{38}O_3$ requires: C, 73·57; H, 11·73%). Levantan-8α, 12,15-triol was also obtained by catalytic hydrogenation of levanten-8α 12,15-triol.

Reaction of α-levantenolide with hydrazine

A mixture of 100 mg of α-levantenolide and 1 ml of 100% hydrazine hydrate in 1 ml of benzene was heated in a boiling water bath. After evaporation of the benzene, heating was continued for 4 hr. Evaporation of the mixture gave 107 mg of solid, m.p. 212–213°. The product was partitioned between ten layers of hexane and ten layers of 70% aqueous methanol. Methanol layers 1–2 yielded 96 mg of 3-(2′-*hydroxy*-2′,5′,5′,8′*a-tetramethyldecahydronaphthalene*-1)*methyl*-4-*methyl*-6-*pyridazone*, m.p. 212–213°, λ_{max} 300, 315 mμ, λ_{min} 265 mμ, $\lambda_{infl.}$ 230 mμ (Found: C, 71·19; H, 9·34; N, 9·21. $C_{20}H_{32}N_2O_2$ requires: C, 72·25; H, 9·70; N, 8·43%). Attempts to recrystallize the pyridazone from several solvents resulted only in the formation of gels, thus preventing further purification. The same pyridazone was also obtained from the reaction of β-levantenolide and hydrazine.

Reaction of α-levantanolide with hydrazine

α-Levantanolide (539 mg) treated with hydrazine hydrate as described above gave 565 mg of crude product. Recrystallization from ethyl acetate gave 3-(2′-*hydroxy*-2′,5′,5′,8′*a-tetramethyldecahydronapthalene*-1)*methyl*-4-*methyl*-4,5-*dihydro*-6-*pyridazone*, m.p. 206–207° (Found: C, 71·53; H, 10·19; N, 8·70. $C_{20}H_{34}N_2O_2$ requires: C, 71·81; H, 10·24; N, 8·38%). This same dihydropyridazone was also obtained by reaction of β-levantanolide with hydrazine hydrate.

Saponification and relactonization of β-levantenolide

A solution of 50 mg of β-levantenolide in 5 ml of methanolic 10% KOH was heated at reflux for five hours. The solution was diluted to 75 ml with water and carefully acidified to pH 4·0 with 1 N H_2SO_4. Extraction with ether gave 53 mg of a gummy acid. The acid was lactonized by heating at 105° for 68 hr. Examination of the infrared spectrum of the product showed that it was predominantly α-levantenolide containing some β-levantenolide.

The above experiment was repeated with α-levantenolide. The product was isolated and identified by its infrared spectrum as α-levantenolide.

Oxidation of α-levantenolide ozonide. Isolation of lactone (IV) *and oxalic acid*

A solution of 200 mg of α-levantenolide in 50 ml of ethyl acetate was cooled in an ice-salt bath. Ozonized oxygen was bubbled through the solution for 2·5 hr. The ozonide obtained after evaporation of the ethyl acetate was oxidized with 60 ml of 3% hydrogen peroxide.[15] The reaction mixture was separated into water-soluble and chloroform-soluble fractions.

The chloroform fraction was chromatographed on silicic acid with benzene and 49:1 benzene–ether to give 123 mg (78·5% yield) of the lactone (IV), m.p. 125–125·5°, $[\alpha]_D^{25}$ +46·5° (C = 1·9 in $CHCl_3$). Ruzicka[9] gives m.p. 123–124°, $[\alpha]_D^{25}$ +45·9° ($CHCl_3$). Identification was confirmed by

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[14] R. F. Nystrom and W. G. Brown, *J. Amer. Chem. Soc.* **69**, 1197 (1947).

[15] 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).

comparison of the infrared spectrum with that of the authentic lactone (IV) and by reduction with lithium aluminum hydride to the glycol,[4] m.p. 132–133°.

The water-soluble fraction was analyzed by paper chromatography with 3:1 phenol-1% formic acid.[16] A single spot, R_f 0·19, was observed in good agreement with oxalic acid.

Reduction of α-levantenolide ozonide. Isolation of the triol (VI)

Following ozonization of 204 mg of α-levantenolide in 50 ml of ethyl acetate at −10°, the ozonide in 20 ml of ether was reduced with 215 mg of lithium aluminum hydride.[13] Chromatography of the crude product on alumina with 19:1 chloroform–ethanol gave 88 mg. of the diol (V), m.p. 132°. Further elution with 9:1 chloroform–ethanol gave 84 mg of the triol (VI), m.p. 159·5–160·5° (Found: C, 71·99; H, 11·52; Act. H, 0·87. $C_{18}H_{34}O_3$ requires: C, 72·43; H, 11·48; Act. H, 1·01%).

Lead tetraacetate oxidation of the triol, m.p. 140–142°

A solution of 84 mg of the triol (VI) and 150 mg of lead tetraacetate in 10 ml of acetic acid was kept for 20 hr at 25°, then diluted with water and steam distilled. The distillate was collected in 100 ml of 2 N hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The resultant precipitate (47 mg), recrystallized from ethanol, gave acetaldehyde 2,4-dinitrophenylhydrazone, m.p. 168–169°. The infrared spectrum was identical with that of an authentic sample.

Wolff–Kishner reduction of the dihydropyridazone

A solution of the crude dihydropyridazone (from 550 mg of β-levantenolide) and 296 mg of potassium hydroxide in 5 ml of diethylene glycol[6] was heated at 100° for three hours, then at 200–210° for 16 hr. The mixture was cooled, then partitioned using five 10% aqueous acetic acid and five aqueous lower layers, and ten benzene upper layers. Benzene layers 0–6 were concentrated and the residue esterified with excess diazomethane.[17] Chromatography of the methyl esters on silicic acid with benzene and 19:1 benzene–ether gave 178 mg of hydroxy ester, m.p. 57–59°. Using a second silicic acid column 105 mm × 45 mm (dia.), the hydroxy methyl ester was chromatographed with 49:1 benzene–ether and 100 ml fractions were collected. Fractions 40–47 gave 70 mg of solid, m.p. 69–72°. Two recrystallizations from petroleum ether gave methyl 13-epilabdanolate, m.p. 71–72°; Bory and Lederer[7] report m.p. 74–75°. The infrared spectrum was identical with that reported in the literature.[7] Fractions 53–59 gave 30 mg of solid, m.p. 65–69°. Two recrystallizations from petroleum ether gave methyl labdanolate, m.p. 72–73°. The melting point was undepressed on admixture with authentic methyl labdanolate isolated from gum labdanum.[18] The infrared spectra of the two specimens were identical.

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[16] J. B. Stark, A. E. Goodban and H. S. Owens, *Analyt. Chem.* **23**, 413 (1951).

[17] Th. J. DeBoer and H. J. Backer, *Org. Syn.* **36**, 16 (1956).

[18] J. D. Cocker, T. G. Halsall and A. Bowers, *J. Chem. Soc.* 4259 (1956).