The Structure of a New Terpenoid Acid, 6(S)-Isopropyl-3 ξ -methyl-3 ξ -hydroxy-9-oxo-4E-decenoic Acid, Isolated from Turkish Tobacco[†]

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The structure of a new hydroxy terpenoid acid, 6(S)-isopropyl-3 ξ -methyl-3 ξ -hydroxy-9-oxo-4E-decenoic acid (1a), isolated from a solvent-extract of Turkish tobacco, was elucidated by the spectroscopic properties and chemical evidence. This compound was suggested to be derived from thunberganoid diterpenoids and be a precursor of two dienoic acids, 6(S)-isopropyl-3-methyl-9-oxo-2E,4E-decadienoic acid (3a) and 6(S)-isopropyl-3-methyl-9-oxo-2Z,4E-decadienoic acid (4a) in Turkish tobacco.

A large number of nor-isoprenoids, the carbon skeletons of which are related to these of thunberganoid diterpenoids are present in tobacco as important constituents of tobacco aroma.^{1~4)}

Studies on acidic aroma constituents of Turkish tobacco in our laboratory led to the isolation of 6(S)-isopropyl-3-methyl-9-oxo-2E,4E-decadienoic acid (3a) and 6(S)-isopropyl-3-methyl-9-oxo-2Z,4E-decadienoic acid (4a)^{4~6)} and these two dienoic acids, geometrical isomers at C(2), was postulated to be formed through a hydroxylated terpenoid acid derived from 1(S)-thunberga-2E,6,11E-triene-4,8-diol⁷⁾ (5), a major diterpenoid component in tobacco leaves.

In this paper, we wish to describe the isolation and structure elucidation of a new constituent which is a possible precursor of these two dienoic acids.

The acidic fraction-33 of the tobacco extract was treated with CH_2N_2 to afford the methyl ester (1b) of the new acidic compound (1a). The molecular formula of the compound (1b) was established to be $\text{C}_{15}\text{H}_{26}\text{O}_4$ by accurate mass determinations of m/e 252 ($\text{M}-\text{H}_2\text{O}$), 239 ($\text{M}-\text{OCH}_3$) and 234 ($\text{M}-2\text{H}_2\text{O}$). The four oxygen atoms in the molecule were respective-

ly assigned, on the basis of the IR, PMR and chemical evidences to be one oxygen atom in a methyl ketone group (1715 cm^{-1} , δ 2.06 (3H, s), positive 2,4-dinitrophenylhydrazine reaction, positive iodoform reaction), two oxygen atoms in a methyl ester group (1735 cm^{-1} , δ 3.69 (3H, s)) and one oxygen atom in a tertiary hydroxy group (3500 cm^{-1} ,

FIG. 1.

[†] The Aroma Constituents of Turkish Tobacco. Part II.⁴⁾

positive Denigés reaction⁸⁾). The PMR spectrum revealed the presence of two methyls in an isopropyl group (δ 0.90 (6H, dd), δ 1.78 (1H, m), 1384, 1365 cm^{-1}) and a methyl attached to the tertiary carbinol group (δ 1.26 (3H, s)).

The PMR signal at δ 2.50 (2H, s), which showed the largest induced shift on the addition of the shift reagent, $\text{Eu}(\text{fod})_3$, indicated the presence of an α -methylene of the methyl ester group linked to the tertiary carbinol group.

As another substituent of this carbinol group, the presence of a *trans* disubstituted double bond attached to the carbon bearing a single proton, was indicated by the IR band at 970 cm^{-1} and the PMR signal at δ 5.39 (2H, m), which was resolved into a doublet (r (relative induced shift ratio⁹⁾)=0.90, J =16 Hz) and a double doublet (r =0.73, J =8, 16 Hz) after the addition of the shift reagent.

The partial structure deduced from the above data, $\text{>CH-CH=CH-C(CH}_3\text{)(OH)-CH}_2\text{-COOCH}_3$, was confirmed by the accurate mass determinations of m/e 117 ($\text{C}_5\text{H}_9\text{O}_3$) and 143 ($\text{C}_7\text{H}_{11}\text{O}_3$) which were caused by the cleavage of an allylic C(3)-C(4) bond and C(5)-C(6) bond, respectively.

The remaining part $\text{C}_7\text{H}_{14}\text{O}$ of the compound (1b) was assigned to consist of the isopropyl group, C_3H_7 , and a 3-oxo-butyl moiety, $\text{CH}_3\text{COCH}_2\text{CH}_2\text{-}$, which was suggested by the PMR signals at δ 1.68 (2H, m) and δ 2.28

(2H, t, J =8 Hz).

Furthermore, the presence of 6-oxo-3-isopropyl-1E-heptenylidene moiety, $\text{CH}_3\text{COCH}_2\text{CH}_2\text{-CH(CH(CH}_3\text{))}_2\text{-CH=CH-}$, was confirmed by the isolation of (+)-2-isopropyl-5-oxo-hexanal (2) on ozonolytic degradation. According to the results of Aasen *et al.*,^{2a)} (+)-2-isopropyl-5-oxo-hexanal possess a (S)-configuration at the carbon carrying an isopropyl group; therefore the absolute configuration at C(6) of the compound (1b) was established to be (S).

The final confirmation of the structure was attained by dehydration. The treatment of the compound (1b) with aqueous $(\text{COOH})_3$ ¹⁰⁾ afforded two dienoates, methyl 6(S)-isopropyl-3-methyl-9-oxo-2E,4E-decadienoate (3b) and methyl 6(S)-isopropyl-3-methyl-9-oxo-2Z,4E-decadienoate (4b), which have been isolated and identified in our previous studies.^{4~6)} From the above data, 6(S)-isopropyl-3 ξ -methyl-3 ξ -hydroxy-9-oxo-4E-decenoic acid was assigned as the structure of the compound (1a).

The presence of 1(S)-isopropyl-2E-propenylidene moiety, $\text{-CH(CH}_3\text{))}_2\text{CH=CH-}$, in the molecule indicates that the compound (1a) may be derived from thunberganoid diterpenoids which are abundant in tobacco.¹¹⁾ The hydroxylated nor-isoprenoid (1a) may have arisen from a hydroxylated thunberganoid, 1(S)-thunberga-2E,6,11E-triene-4,8-diol (5) through the oxidative cleavage of C(6)-C(7) and C(11)-C(12) bonds. Further oxidative degradation of the compound (1a) during curing and ageing of tobacco leaves may give a lot of anomalous nor-isoprenoids with fewer carbon atoms.^{3a, 4, 12)}

Because of a shortage of the material (1b),

the absolute configuration at C(3) could not be determined. The PMR spectrum obtained in the presence of $\text{Eu}(\text{fod})_3$ failed to show any contamination of the C(3)-epimer in the isolated material (1b). However, it may be reasonably expected that the epimer is present in the cured leaves since the corresponding precursor, the C(4)-epimer of the compound (5), has been isolated from the leaves.⁷⁾

These nor-isoprenoids related to thunberganoids in general have a gentle green aroma reminiscent of vegetables, whereas thier precursors, thunberganoids, do not possess any characteristic aroma. The possible formation of these nor-isoprenoids during the curing and ageing of tobacco leaves in the presence of light and oxygen may be important for the improvement of the quality of the leaves by those processings.

EXPERIMENTAL

PMR spectra, IR spectra and optical rotations were measured on a JNM-PS-100 instrument, a JASCO IR-G instrument and a JASCO DIP-181 instrument, respectively. Accurate mass determinations were carried out on a HITACHI RMU-7 instrument operated at 70 eV and GC-MS was recorded on a HITACHI RM-50GC instrument using a 20 m $\times$ 0.25 mm glass capillary column coated with OV-101 under the following conditions; split ratio 1:30, carrier gas, He, 1 ml/min, oven temp., 50~195°C, 2°C/min, injection temp., 270°C. Preparative GC was carried out on a HITACHI F6-D instrument using a 1 m $\times$ 3 mm stainless column packed with SE-30 (5%) on Chromosorb W. Each constituent was collected at -70°C in a U-shaped glass tube connected directly to the outlet pipe heated to 280°C.

Isolation of methyl 6(*S*)-isopropyl-3 ξ -methyl-3 ξ -hydroxy-9-oxo-4*E*-decanoate (1b)

Fraction-33 (10.02 g) of the acidic fraction of the

isopropyl ether extract of cured Turkish tobacco (IZUMIR U) as described previously,⁴⁾ was esterified with CH_3N_2 and the material was separated on a silicic acid column using a hexane-ether mixture as a solvent. The methyl ester (1b, 30 mg) of the hydroxy acid (1a) was isolated from subfraction-8 (hexane: ether=30:70) by the preparative GC: $[\alpha]_D^{25} -3.8$ ($c=1.0$ in CH_3OH); MS (70 eV); M^+ at 270 was not seen, 43 (100), 97 (30), 95 (27), 55 (23), 53 (22), 67 (22), 81 (14), 93 (12), 111 (9.6), 71 (9.6), 69 (9.6), 79 (9.3), 77 (9.0), 121 (7.2), 123 (6.9), 135 (6.9), 84 (6.9), 125 (4.8), 107 (4.5), 109 (4.5), 85 (4.2), 120 (4.0), 110 (3.5), 99 (3.5), 234 (3.0), 153 (2.0), 178 (2.0), 154 (1.8), 117 (1.5), 181 (1.5), 239 (1.1), 143 (1.0), 252 (0.5).

Accurate mass measurements, $\text{C}_{15}\text{H}_{24}\text{O}_3$ ($\text{M}-\text{H}_2\text{O}$), found 252.1704; calcd. 252.1723, $\text{C}_{14}\text{H}_{23}\text{O}_3$ ($\text{M}-\text{OCH}_3$), found 239.1609; calcd. 239.1646, $\text{C}_{15}\text{H}_{22}\text{O}_2$ ($\text{M}-2\text{H}_2\text{O}$), found 234.1624; calcd. 234.1618, $\text{C}_{10}\text{H}_{17}\text{O}$, found 153.1281; calcd. 153.1279, $\text{C}_7\text{H}_{11}\text{O}_3$, found 143.0722; calcd. 143.0707, $\text{C}_8\text{H}_{10}\text{O}$, found 127.1104; calcd. 127.1122, $\text{C}_8\text{H}_6\text{O}_3$, found 117.0580; calcd. 117.0551. IR (cm^{-1} , film), 3500 (broad), 2950 (s), 2930 (m), 2870 (m), 1735 (s), 1715 (s), 1437 (m), 1384 (w), 1365 (m), 1337 (m), 1205 (s), 1165 (m), 1115 (m), 1009 (m), 970 (m), 936 (w); PMR (δ , CCl_4), 0.90 (6H, dd, $J=7$ Hz), 1.26 (3H, s), 1.2~1.8 (4H, m), 2.06 (3H, s), 2.28 (2H, t, $J=8$ Hz), 2.50 (2H, s), 3.69 (3H, s), 5.39 (2H, m); Irradiations at δ 1.62, δ 1.68 and δ 1.78 simplified the multiplet at δ 5.39 to a sharp singlet, the triplet at δ 2.28 to a broad singlet and the double doublet at δ 0.90 to a broad singlet: Addition of shift reagent, $\text{Eu}(\text{fod})_3$, r (relative induced shift ratio⁹⁾) = 0.15 ($-\text{CH}(\text{CH}_3)_2$, dd), $r=0.21$ (COCH_3 , s), $r=0.25$ (COOCH_3 , s), $r=0.34$ ($-\text{CH}-\text{C}=\text{C}-$, m), $r=0.73$ ($-\text{CH}=\text{C}-\text{C}(\text{OH})-$, dd, $J=8, 16$ Hz), $r=0.90$ ($-\text{C}=\text{CH}-\text{C}(\text{OH})-$, d, $J=16$ Hz), $r=1.00$ ($\text{C}(\text{CH}_3)(\text{OH})$, s), $r=1.23$ ($-\text{C}(\text{OH})-\text{CH}_2-\text{COOCH}_3$, m): Irradiation at the signal with $r=0.34$ simplified the double doublet signal with $r=0.73$ to a doublet ($J=16$ Hz).

Dehydration of methyl 6(*S*)-isopropyl-3 ξ -methyl-3 ξ -hydroxy-9-oxo-4*E*-decanoate (1b)

The ester (1b, 1 mg) was treated with 6% aqueous oxalic acid (2 ml) at 70°C for 2 hr.¹⁰⁾ After cooling, the ether-soluble part was subjected to GC-MS, which showed three peaks. Their mass fragment patterns

were respectively indistinguishable from that of solanone¹⁾ (5(S)-isopropyl-8-methyl-6E,8-nonadien-2-one),^{2a)} methyl 6(S)-isopropyl-3-methyl-9-oxo-2E,4E-decadienoate (3b) and methyl 6(S)-isopropyl-3-methyl-9-oxo-2Z,4E-decadienoate (4b). (The isolation of 6(S)-isopropyl-3-methyl-9-oxo-2Z,4E-decadienoic acid (4a) was previously reported without the assignment of the C(6)-chirality.⁴⁾ The absolute configuration of C(6) was established to be (S) on the ozonolytic degradation as described in the experimental.) These three compounds were not separated from the authentic samples when co-injected on a glass capillary GC column.

Ozonolysis of methyl 6(S)-isopropyl-3ξ-methyl-3ξ-hydroxy-9-oxo-4E-decenoate (1b)

The ester (1b, 20 mg) dissolved in CH₂Cl₂ (15 mg) was treated with excess ozone at -60°C. Zinc-powder (500 mg) and acetic acid (2 ml) were then added to the solution which was stirred for 30 min, while the temperature was slowly raised to room temp. The reaction solution was washed with water four times and a slightly yellow oil was obtained after the removal of the solvent. Chromatography of this material on a silicic acid column furnished pure 2(S)-isopropyl-5-oxo-hexanal (2, 1.5 mg); its spectral data (GC-MS, PMR) were identical to those reported by Aasen *et al.*^{2a)}; [α]_D²⁵ 40.0 (589 nm), 40.0 (577 nm), 66.7 (546 nm), 106.7 (435 nm), 333.3 (365 nm) (c=0.075 in CH₃OH).

Ozonolysis of methyl 6(S)-isopropyl-3-methyl-9-oxo-2Z,4E-decadienoate (4b)

The ester (4b, 10 mg) gave pure 2(S)-isopropyl-5-oxo-hexanal (2, 0.9 mg) on the ozonolytic degradation by a similar method as above; [α]_D²⁵ 44.4 (589 nm), 44.4 (577 nm), 66.7 (546 nm), 111.1 (435 nm), 311.1 (365 nm) (c=0.045 in CH₃OH).

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