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The Characterization of Malloprenol and Its Ester with Linolenic Acid Isolated from the Leaves of *Mallotus japonicus*

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An isoprenoid alcohol (I) and the ester with linolenic acid (II) isolated from *Mallotus japonicus* of *Euphorbiaceae* were investigated. The trivial name "mallophenol" was proposed to describe the prenol (I). Nuclear magnetic resonence studies showed that the prenol contained one *trans* and six *cis* internal isoprene residues, and a *cis* 'OH-terminal' isoprene residue.

Mallotus japonicus is a deciduous tree of the *Euphorbiaceae* which has long been used in Japan as a gastrointestinal drug. In the studies of this plant, the presence of bergenin,[1] rutin,[2] and some fatty acids,[3] but not linolenic acid, has been reported.

From the nonpolar fractions of the methanol extracts of leaves which has been collected in Ohito and Nagaoka-cho, Shizuoka Prefecture, in September and October, 1968, malloprenol (I) and the ester with linolenic acid (II) were isolated. The nonpolar fractions of the extracts were then chromatographed on silica gel to give malloprenol and the ester with linolenic acid, which was less polar than the malloprenol (I). The malloprenol (I) is a colorless oil, $n_D^{23.5}$ 1.5038. The molecular formula, $C_{45}H_{74}O$ (mol wt, 631.0), was deduced from the analytical data and from the molecular weight*1 of (I) (the obsd mol wt, 652) and its derivatives. The IR spectra showed absorptions at 3325, 1005, and 1665 cm^{-1}, thus confirming the presence of a primary hydroxyl group and isolated double bonds. In the UV spectrum, there was no absorption longer than 210 mμ. The malloprenol (I) gave the acetate (III), with acetic anhydride-pyridine, $C_{47}H_{76}O_2$ (mol wt, 673.28, obsd mol wt, 669, 667), or (IV) with *p*-bromobenzoylchloride-pyridine, $C_{52}H_{77}O_2Br$ (mol wt, 814.05; obsd mol wt, 854). In the IR spectra there was no hydroxyl band, but there did appear a strong absorption of the carbonyl group at 1740 cm^{-1} in (III) and at 1730 cm^{-1} in (IV). The primary hydroxyl group in (I) was easily oxidized with the chromium trioxide-pyridine complex to give the aldehyde (V), $C_{45}H_{72}O$ (mol wt, 629.02; obsd mol wt 645). In the IR spectrum, there were absorptions of the formyl group (2740 and 1670 cm^{-1}) and the conjugated double bond (1620 cm^{-1}). The formyl proton appeared at 9.90 ppm (1H, d, J=8Hz) in the NMR spectrum. The strong absorption at 231 mμ in the UV spectrum confirmed that Compound I was a primary allylic alcohol. Compound V gave the 2,4-dinitrophenylhydrazone as a reddish oil, $C_{51}H_{76}O_4N_4$ (mol wt, 809.15; obsd mol wt, 833). From the ozonization of the malloprenol (I), followed by the reductive decomposition of the ozonide, levulinaldehyde and acetone were isolated as their 2,4-dinitrophenylhydrazones. From the above results, it appeared that malloprenol was an isoprenoid alcohol.

The NMR spectrum of (I) resembles those of solanesol[4] (all *trans*) and castaprenol-11, -12 and -15.[5] There are two peaks at 1.60 and 1.68 ppm with a shoulder at 1.73 ppm in Compound I. The relative areas under the two peaks are about six and twenty-four protons (including the shoulder at 1.73 ppm) respectively. The peak at 1.60 ppm can be assigned to two methyl groups of one internal and one ω-terminal *trans* isoprene residues. The eight methyl group at 1.68 ppm show the seven methyl groups of the six internal and one ω-*cis* isoprene residues and one methyl group of the *cis* 'OH-terminal' isoprene residue (as shoulder at 1.73 ppm.[5]) The olefinic protons give a broad peak at 5.13 ppm (8H) and a triplet at 5.42 ppm (1H, J=7Hz), corresponding to the proton on the β-carbon to the hydroxyl-group coupling with the α-methylene protons to the hydroxyl group, which appear at 4.11 ppm (2H, J=7Hz) in the malloprenol (I).

From the above observations, the prenol can be said to contain a *cis* 'OH-terminal' isoprene residue

1) K. Shibata and M. Shimogori, *Nippon Kagaku Zasshi*, **70**, 36 (1949).

2) T. Kashimoto and K. Noda, *ibid.*, **79**, 873 (1958).

3) K. Honma, Japan. 207849 (1954).

*1 The molecular weights were measured by the osmotic method on a Hitachi Perkin-Elmer Molecular-weight Apparatus 115 in a benzene solution.

4) R. L. Rowland, P. H. Latimer and J. A. Giles, *J. Amer. Chem. Soc.*, **78**, 4680 (1956).

5) A. R. Wellburn, J. Slevenson, F. W. Hemming and R. A. Morton, *Biochem. J.*, **102**, 313 (1967).

(shoulder at 1.73 ppm), one internal *trans* isoprene residue (1.60 ppm), and, of necessity, methyl groups both *cis* and *trans* to the olefinic proton in the ω-isoprene residue (*cis*: 1.68 ppm; *trans*: 1.60 ppm). Six of the other isoprene rdsidues are internal *cis* (1.68 ppm). In Compounds III and IV, the metyl groups of the *cis* 'OH-terminal' isoprene residue appeared at 1.75 and 1.77 ppm respectively. The allylic methylene protons appeared as two peaks, at 2.02—2.05 and 2.07—2.08 ppm, in Compounds (I—IV).

$$H\text{-}(CH_2\text{-}\overset{\overset{\large CH_3}{|}}{C}{=}CH\text{-}CH_2)_9\text{-}OR$$

I: R=H

II: $R{=}CH_3(CH_2\text{-}CH{=}CH)_3\text{-}CH_2(CH_2)_6\cdot CO\text{-}$

The ester (II), $n_D^{19.5}$ 1.5057, showed strong absorptions at 1740 and 1170 cm^{-1}, but no hydroxyl band in its IR spectrum. The alkaline hydrolysis of the ester gave the components, an alcohol, and an acid. The NMR and IR spectra of the alcohol were the same as those of the malloprenol (I) in all respects. The acid afforded the methyl ester (VI) with diazomethane. The ester (VI) (M^+/e 292) was identified as methyl linolenate (mol wt, 292.44) by a study of its GLC, IR, and NMR spectra.

Experimental

The IR spectra were recorded on a Hitachi Grating Infrared Spectrometer EPI G-2; the UV spectra, on a Hitachi Recording Spectrophotometer EPS-3, and the NMR spectra, on a Hitachi Nuclear Magnetic Resonance Spectrometer R-20. The chemical shifts were measured on the δ scale relative to TMS as the internal standard (δ=0).

Extraction and Isolation of Malloprenol I and the Ester with Linolenic Acid (II). Dried leaves (5.1 kg) were extracted with methanol (75 *l*) at room temperature for two weeks. The methanol extract was then concentrated to 5 *l*, and water (5 *l*) was added. The solution was subsequently extracted with light petroleum ether. The evaporation of the solvent left a tarry residue (205 g), which was then chromatographed on silica gel. Ligroin/benzene (6:1) eluted fractions which gave dark brown spots (R_f 0.7) with a concentrated sulfuric acid spray on chromatoplates developed with ligroin/benzene (4:1). The fractions were combined and evaporated, and the residue was repeatedly chromatographed to give a colorless oil, (II) (1.7 g), which showed a single spot on TLC, $n_D^{17.5}$ 1.5057; Found: C, 84.85; H, 11.51%. Calcd for $C_{63}H_{102}O_2$: C, 84.88; H, 11.53%. IR: 3020, 1665, 840 (C=C), 1740, 1170 cm^{-1} ($OCOCH_3$). $\delta_{ppm}^{CDCl_3}$ 0.98 (3H, t, J=7.5 Hz, $C\underline{H}_3CH_2$–), 1.33 [10H, $(\text{–}C\underline{H}_2)_5\text{–}CH_2COO$–], 1.60(6H, $C\underline{H}_3{>}C{=}C{<}H$), 1.67 (24H, $C\underline{H}_3{>}C{=}C{<}H$), 2.03—2.08(32H, $\text{–}C\underline{H}_2\text{–}C{=}CH$), 2.08(4H, $C\underline{H}_2\text{–}CH{=}CH$), 4.55(2H, d, J=7.5 Hz, C=CH–$C\underline{H}_2$–OH), 5.13(8H, C=C$\underline{H}$–CH_2).

Benzene subsequently eluted fractions which gave dark brown spots (R_f 0.4) with a concentrated sulfuric acid spary on chromatoplates developed with benzene. After the evaporation of the solvent, the residue was rechromatographed several times to give a colorless oil, (I) (30 g), which showed a single spot on reversed-phase partition TLC on paraffin-impregnated kieselgel (impregnated with 5%, v/v, liquid paraffin in petroleum ether) with acetone - water (23 : 2, v/v) saturated with paraffin as the mobile phase,[6] $n_D^{23.5}$ 1.5038. Found: C, 85.70; H, 11.72%; mol wt, 652. Calcd for $C_{45}H_{74}O$; C, 85.64; H, 11.82%; mol wt, 631.04. IR: 3325, 1090 (OH), 3025, 1665, 840 (C=C), 2970, 2925, 2850, 1450, 1375 cm^{-1} (–CH_2–, –CH_3). $\delta_{ppm}^{CDCl_3}$: 1.31 (1H, –$CH_2O\underline{H}$), 1.60 (6H, $C\underline{H}_3{>}C{=}C{<}H$), 1.68 (24H, $CH_3{>}C{=}C{<}H$), 2.02 and 2.07 (32H, –$C\underline{H}_2$–CH=C), 4.06 (2H, d, J= 7 Hz, C=CH–$C\underline{H}_2$–OH), 5.13 (8H, C=C$\underline{H}$–CH_2–), 5.42 (1H, t, J=7 Hz, C=C$\underline{H}$–CH_2–OH).

Monoacetate (III). The malloprenol (I) (590 mg) in 0.5 m*l* of pyridine was treated with 0.5 m*l* of acetic anhydride and then allowed to stand overnight. To the reaction mixture 15 m*l* of water were then added, and the oily product was extracted with benzene to give 522 mg of the crude acetate. Using silica-gel chromatography and elution with petroleum ether/benzene (1 : 1), 485 mg of III was obtained; $n_D^{22.5}$ 1.5047. Found: C, 83.52; H, 11.32%; mol wt, 669, 667. Calcd for $C_{47}H_{76}O_2$: C, 83.86; H, 11.38%; mol wt, 673.08. IR: 3025, 1660, 835 (C=C), 1740, 1230 cm^{-1} ($OCOCH_3$).

***p*-Bromobenzoate (IV).** The malloprenol (I) (500 mg) in 1.5 m*l* of pyridine was treated with 510 mg of *p*-bromobenzoyl chloride and then allowed to stand overnight at room temperature. To the reaction mixture, 20 m*l* of benzene were then added, after which the precipitate was filtered off and the filtrate was washed with a $NaHCO_3$ solution, 0.5N HCl, and water. After the removal of the solvent, the residue (581 mg) was chromatographed on silica gel (20 g) to give 508 mg of (IV); $n_D^{22.5}$ 1.5267. Found: C, 77.20; H, 9.73; Br, 9.70%; mol wt, 854. Calcd for $C_{52}H_{77}O_2Br$: C, 76.72; H, 9.73; Br, 9.82%; mol wt, 814.05. IR: 3030, 1665, 850 (C=C), 1730, 1590, 1270, 760 cm^{-1} (benzyl).

The Aldehyde V and Its 2,4-dinitrophenylhydrazone. To a CrO_3-pyridine complex (CrO_3 1 g, pyridine 10 m*l*) we added 1.5 g of I in 6 m*l* of pyridine with cooling at 0°; the mixture was then allowed to stand for an hour at room temperature. The reaction mixture was filtered, and the dark brown solid remaining was washed with pyridine. The combined pyridine solution was diluted with water and extracted with benzene. After drying (Na_2SO_4), the solvent was removed and the residue was chromatographed on silica gel (35 g) to give 975 mg of V; $n_D^{22.5}$ 1.5126. Found; C, 85.77; H, 11.46%; mol wt, 645. Calcd for $C_{45}H_{72}O$: C, 85.92; H, 11.54%; mol wt, 629.02. IR: 2740, 1670, 1620 cm^{-1} (C=C–CHO). λ_{max} 231 mμ (ε=1.38×10^4 in cyclohexane). $\delta_{ppm}^{CDCl_3}$ 5.86 (1H, d, J=8 Hz, C=C$\underline{H}$–CHO), 9.90 (1H, d, J=8 Hz, C=CH–C$\underline{H}$O). To 156 mg of (V), 2,4-dinitrophenylhydrazine in an EtOH solution was added; after dilution with water, a reddish oily product was extracted with benzene. After the removal of the solvent, the residue (195 mg) was chromatographed on silica gel to give 166 mg of the 2,4-dinitrophenylhydrazone.

Found: C, 75.64; H, 9.51; N, 6.57%; mol wt, 833. Calcd for $C_{51}H_{76}O_4N_4$: C, 75.70; H, 9.47; N, 6.92%; mol wt, 809.15.

6) K. J. Sone, A R. Wellburn, F. W. Hemming and J. F. Pennock, *ibid.*, **102**, 325 (1967).

Ozonolysis of the Malloprenol (I). A solution of 1.554 g of the malloprenol (I) in 150 m*l* of ethyl acetate was ozonized at −70°C until the solution became blue. The solvent was then removed *in vacuo* to give the ozonide as a pale yellow oil. To the ozonide 200 m*l* of water and 15 g of zinc dust were added and then decomposed by heating them at 95°C for 8 hr. To this reaction mixture, nitrogen gas was blown and the volatile component was collected as its 2,4-dinitrophenylhydrazone (64 mg; mp 117—119°C).

Found: C, 45.23; H, 3.99; N, 23.33%; Calcd for $C_9H_{10}N_4O_4$: C, 45.38; H, 4.23; N, 23.52%. No depression of mp was noted with an authentic sample of acetone 2,4-dinitrophenylhydrazone. After the filtration of the zinc dust, 500 m*l* of 2N HCl saturated with 2,4-dinitrophenylhydrazine was added to the filtrate. The precipitate was collected and crystallized from dimethylformamide to give 559 mg of fine needles; mp 245—246°C. Found: C, 44.25; H, 3.28; N, 24.17%. Calcd for $C_{17}H_{16}O_8N_8$: C, 44.35; H, 3.50; N, 24.34%. No depression of mp was noted with an authentic sample of levulinaldehyde 2,4-dinitrophenylhydrazone.

Alkaline Hydrolysis of II.] To the solution of II (600 mg) in 20 m*l* of benzene we added 20 m*l* of alcoholic 1N KOH, after which the mixture was refluxed for 4 hr. The organic solvents were then evaporated, leaving a residue which was dissolved in 30 m*l* of water and extracted with light petroleum ether to give a colorless oil (498 mg). The oil was identical with (I) in all respects. The aqueous solution was acidified with 1N HCl and extracted with ether to give 114 mg of a colorless oil which was esterified with diazomethane to give 105 mg of (VI); M^+/e 292. IR: 3025, 1650, 725 (C=C), 1745, 1175 cm^{-1} ($COOCH_3$). $\delta^{CDCl_3}_{ppm}$ 0.97 (3H, t, J=6.8 Hz, $C\underline{H}_3CH_2$), 1.27—1.33 [10H, $-(C\underline{H}_2)_5-CH_2COOCH_3$], 2.81 (4H, $C\underline{H}_2-CH=CH$), 3.67 (3H, s, $COOC\underline{H}_3$), 5.3—5.5 [6H, $(CH_2-C\underline{H}=C\underline{H})_3$].

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