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TERPENOIDS AND RELATED COMPOUNDS—XI[1]

CHEMICAL INVESTIGATION OF *ALEURITES MONTANA* AND THE STRUCTURE OF ALEURITOLIC ACID—A NEW TRITERPENE ACID*

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Abstract—The isolation of friedelin, β-sitosterol, betulinic acid and a new triterpene acid, aleuritolic acid, $C_{30}H_{48}O_3$ from the bark of *Aleurites montana* has been described. The new acid is present as its acetate. On the basis of physical and chemical evidences, structure IV is suggested for aleuritolic acid.

Aleurites montana[2] (Euphorbiaceae) is a tree of moderate height called Tung in Bengali.

The benzene extract of the bark of *A. montana* was separated into the acid and the neutral fractions. The neutral fraction on chromatography first yielded friedelin, m.p. 259–61°, $(\alpha)_D$–36°, identical with an authentic sample[3] (m.m.p. and IR). The more polar component, $C_{29}H_{50}O$, m.p.135–7°, $(\alpha)_D$–40° was identified as β–sitosterol.[4] The acid fraction on esterification with diazomethane and subsequent chromatography first yielded the new compound, 3β-acetoxy methyl aleuritolate (I) m.p. 241–43°, $(\alpha)_D$+23.08°. The corresponding acid, named aleuritolic acid (IV) has been shown to be olean-14(15)-en-3β-ol-28-oic acid on the basis of chemical and physical evidence. The more polar component m.p. 220 –22°, $(\alpha)_D$+1·4°, isolated from the chromatogram has been identified as methyl betulinate.[5]

Acetoxy methyl aleuritolate (I) $C_{33}H_{52}O_4$, (M^+, 512), $(\alpha)_D$ + 23·08°, no UV absorption above 220 mμ, ν_{max} 1735 cm^{-1} (broad peak,—O—$COCH_3$ and —$COOCH_3$), 1245 cm^{-1} (—O—$COCH_3$), 820 cm^{-1} (trisubstituted double bond). Its NMR spectrum showed the presence of seven tertiary Me groups between 0·8 to 1·05 ppm, one acetoxy group at 2·04 ppm and one carbomethoxy group at 3·58 ppm. It showed a multiplet centered at 5.50 ppm indicating the presence of a trisubstituted double bond. Hydrolysis of acetoxy methyl aleuritolate (I) with 5% methanolic KOH furnished methyl aleuritolate II, m.p. 208–10°, $(\alpha)_D$ + 11.11°, ν_{max}^{KBr} 3480 cm^{-1} (—OH), 1735 cm^{-1} (—$COOCH_3$), 820 cm^{-1} (trisubstituted double bond), NMR signals at 5·50 ppm (multiplet, 1H, trisubstituted double bond), 3·54 ppm (—$COOCH_3$) and signals for seven tertiary Me groups. Methyl aleuritolate on prolonged treatment with 10% and 15% methanolic KOH under refluxing conditions gave back the starting material indicating the hindered nature of the carboxyl group. Methyl aleuritolate (II) on CrO_3–pyridine oxidation[6] gave the ketoester (III) $C_{31}H_{48}O_3$, (M^+, 468), $(\alpha)_D$ + 11·76°, ν_{max}^{KBr} 1705 cm^{-1} (6-membered ring ketone), 1735 cm^{-1} (trisubstituted double bond), NMR signals at 5·58 ppm (multiplet, 1H, trisubstituted double bond),

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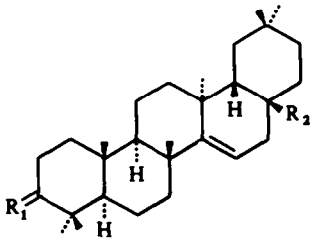


I: $R_1 = \overset{H}{\underset{OAC}{<}}$ $R_2 = COOCH_3$

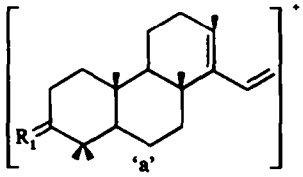
II: $R_1 = \overset{H}{\underset{OH}{<}}$, $R_2 = COOCH_3$

III: $R_1 =$ O, $R_2 = COOCH_3$

IV: $R_1 = \overset{H}{\underset{OH}{<}}$, $R_2 = COOH$

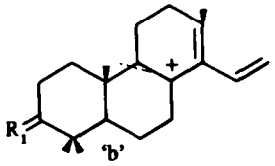
V: $R_1 = \overset{H}{\underset{OH}{<}}$, $R_2 = CH_2OH$

VI: $R_1 = \overset{H}{\underset{OAC}{<}}$ $R_2 = CH_2OAC$



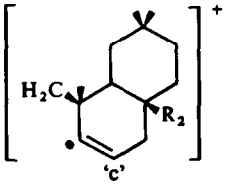
'a'

m/e 344 for I
m/e 300 for II



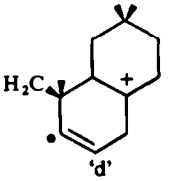
'b'

m/e 329 for I
m/e 285 for III



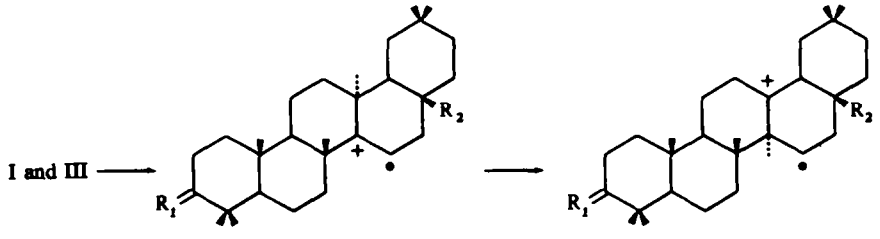
'c'

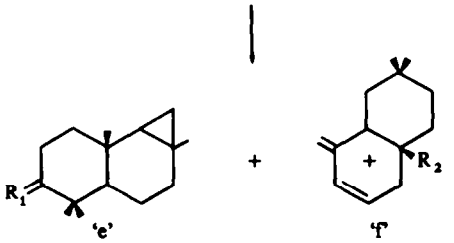
m/e 248 for I and II



'd'

m/e 189 for I and III





'e'

m/e 262 for I
m/e 220 for III

'f'

m/e 233

3·58 ppm (—$COOCH_3$) and signals for seven tertiary Me protons. Hydrolysis of methyl ester II with potassium tertiary butoxide in DMSO[7] furnished the new acid IV, m.p. 300–302° (dec.), $\nu_{max}^{CHCl_3}$ 3420 cm^{-1} (—OH), 1700 cm^{-1} (—COOH), 820 cm^{-1} (trisubstituted double bond) for which the name aleuritolic acid is proposed.

Acetoxy methyl aleuritolate (I) showed yellow coloration with TNM and consumed one mole equivalent of perbenzoic acid indicating the presence of one double bond. Acid isomerization[8] of I gave acetyl methyl oleanolate m.p. 219–20°, $(\alpha)_D$ 58.82°[9] (m.m.p. and IR). This fact coupled with the NMR data establishes that aleuritolic acid (IV) contains a modified oleanane skeleton with a double bond, a β—OH at C—3 and a —COOH group at C—17.

The position of the double bond at 14–15 position in aleuritolic acid (IV) was established from the mass spectral fragmentation pattern of acetoxy methyl aleuritolate (I) and methyl aleuritolonate (III). Compounds I and III exhibited mass peaks 'a' at *m/e* 344 and *m/e* 300 respectively. These ion peaks are accompanied by peaks 15 mass units lower 'b', which are formed by the loss of allylically activated Me group at C–8. The spectrum of I exhibited in addition, a peak at *m/e* 284 ('a'—CH_3COOH) and at *m/e* 269 ('b' —CH_3COOH). In addition to species 'a' and its further decomposition products, the spectra of I and III showed a very abundant peak 'c' at *m/e* 284 derived from rings D and E. Furthermore, fragment 'c' loses the substituent at C–17 giving rise to a fragment 'd' at *m/e* 189, ('c' —$COOCH_3$). The appearance of small but prominent peaks at *m/e* 262 and 220, 'e' derived from compounds I and III respectively and peaks at *m/e* 233, 'f' accompanied by a peak at *m/e* 174 ('f' — $COOCH_3$) can be explained by reactions shown in the accompanying chart. This type of fragmentation is consistent with the mass spectral data of Δ^{14}-taraxerene derivatives reported by Djerassi *et al.*[10]

The above data are compatible with structure IV for aleuritolic acid and co-relation with a suitable member of oleanane series was considered. LAH reduction of acetoxy methyl aleuritolate(I) furnished the corresponding diol (V) m.p. 265–67° which was identified as myricadiol[11–14] (m.m.p. and IR). Acetylation of the diol gave myricadiol diacetate VI, m.p. 251–53°, $(\alpha)_D$–3° identical with an authentic sample of myricadiol diacetate (m.m.p. and IR).

EXPERIMENTAL

M.p's are uncorrected. Petroleum used throughout the investigation had b.p. 60–80°. Optical rotations refer to solns. in $CHCl_3$. IR spectra were recorded in Perkin-Elmer model 337 spectrophotometer. NMR spectra were determined in $CDCl_3$ on a Varian A–60 spectrometer using TMS as internal standard.

Extraction of the bark of A. montana. The dried ground bark of *A. montana* (2 Kg) was extracted with benzene in a Soxhlet apparatus for 18 hr. The gummy residue obtained after the removal of benzene was separated into the acid and neutral fractions. The neutral gummy fraction (4·6 gm) obtained after the evaporation of ether was chromatographed over alumina (200 gm, deactivated with 8 ml of 10% aqueous AcOH). Elution with petroleum yielded a fraction m.p. 244–48° (0·85 gm), which on crystallization from $CHCl_3$–MeOH mixture afforded pure friedelin, m.p. 259–61°, $(\alpha)_D$ –36° identical with an authentic specimen (m.m.p. and IR). (Found: C, 84·18; H, 11·76. Calc. for $C_{30}H_{50}O$: C, 84.44; H, 11·81%). Further elution of the column with petroleum: benzene (3:2) gave a solid (1·21 gm) m.p. 130–32° which on crystallization from $CHCl_3$–MeOH mixture gave pure β-sitosterol, m.p. 135–37°, $(\alpha)_D$ –40°, acetate, m.p. 128–29°, $(\alpha)_D$ –38°, identical with an authentic specimen (m.m.p. and IR). (Found: C, 81·47; H, 11·84. Calc for $C_{31}H_{52}O_2$: C, 81·52; H, 11·48%).

Examination of the acid fraction. The alkali washed portion was acidified with dil HCl and then extracted with ether. On removal of the ether a gummy residue (1·00 gm) was obtained which was esterified with diazomethane. The crude ester (0·60 gm) was chromatographed over a column of alumina (40·0 gm, deactivated with 1·5 ml of aqueous AcOH). Petroleum eluted a semisolid mass (0·30 gm) which on digestion with petroleum afforded crystalline solid (0·11 gm), m.p. 235–38°. The latter on crystallization from $CHCl_3$–MeOH mixture afforded pure I, m.p. 241–43°, $(\alpha)_D$+23·08°. (Found: C, 76·88; H, 10·76. $C_{33}H_{53}O_4$ requires: C, 77·34; H, 10·15%), $\nu_{max}^{CHCl_3}$ 1735 cm^{-1} (broad, —O—$COCH_3$ and —$COOCH_3$), 1245 cm^{-1} (—O—$COCH_3$), 820 cm^{-1} (trisubstituted double bond). Further elution of the column with petroleum: benzene (3:2) gave a solid (0·12 gm) m.p. 216–18°, which after crystallization from $CHCl_3$–MeOH mixture gave the pure solid, m.p. 220–22°, $(\alpha)_D$ + 1·4° identical with an authentic sample of methyl betulinate (m.m.p. and IR). (Found: C, 78·91; H, 10·80. Calc for $C_{31}H_{50}O_3$: C, 79·10; H, 10·71%).

Hydrolysis of acetoxy methyl aleuritolate (I) *and preparation of methyl aleuritolate* (II) To a soln of I (0·20 gm) in benzene (10 ml), 10% methanolic KOH (30 ml) was added and the reaction mixture was refluxed for 4 hr. After usual work up and crystallization from $CHCl_3$–MeOH it gave pure II, m.p. 208–10°, $(\alpha)_D$ + 11·11°, ν_{max}^{KBr} cm^{-1} (—OH), 1735 cm^{-1} (—$COOCH_3$), 820 cm^{-1} (trisubstituted double bond). (Found: C, 78·92; H, 10·56. $C_{31}H_{50}O_3$ reqires: C, 79·10; H, 10·71%).

CrO_3-*Pyridine oxidation of methyl aleuritolate* (II) *and preparation of methyl aleuritolonate* (III). A soln of II (0·20 gm) in pyridine (6 ml) cooled to 15° was added to a CrO_3–Pyridine complex,[6] prepared from CrO_3 (0·20 gm) and pyridine (2 ml) at 15° and the reaction mixture was allowed to stand at room temp for 18 hr. Excess of CrO_3 was decomposed by addition of MeOH (5 ml). The mixture was then digested with EtOAc and filtered. The filtrate after working up as usual, yielded a solid (0·19 gm), which was chromatographed over activated alumina (20 gm). Elution with petroleum afforded a solid (0·12 gm), m.p. 171–74°, which after crystallization from $CHCl_3$–MeOH mixture furnished III (0·08 gm), m.p. 174–76°, $(\alpha)_D$ + 11·76°, ν_{max} 1705 cm^{-1} (six membered ring ketone), 1735 cm^{-1} (—$COOCH_3$), 820 cm^{-1} (trisubstituted double bond). (Found: C, 79·41; H, 10·32. $C_{31}H_{48}O_3$ requires: C, 79·48; H, 10·45%).

Hydrolysis of methyl aleuritolate and preparation of aleuritolic acid (IV). To a normal soln of t-BuOK in t-BuOH (prepared from 0·4 gm of K in 10 ml dry t-BuOH) a soln of methyl aleuritolate (0·15 gm) in DMSO (10 ml) was added and the reaction mixture was heated on an oil bath at 105° for 4 hr.[7] The reaction mixture was then cooled, diluted with water and acidified with dil HCl. The solid that separated out was taken up in $CHCl_3$, which after being washed with water was dried (Na_2SO_4). Removal of the solvent gave an amorphous solid which after crystallization from $CHCl_3$–MeOH mixture gave a solid, m.p. 300–302° (dec), $\nu_{max}^{CHCl_3}$ 3400 cm^{-1} (—OH), 1700 cm^{-1} (COOH), 820 cm^{-1} (trisubstituted double bond). (Found: C, 78·84; H, 10·38. $C_{30}H_{48}O_3$ requires: C, 78·94; H, 10·52%).

Acetyl aleuritolic acid. Aleuritolic acid IV on acetylation by Ac_2O–Pyridine method gave the acetyl derivative which on crystallization from $CHCl_3$–MeOH mixture gave crystalline acetyl aleuritolic acid m.p. 278–81°. (Found: C, 79·32; H, 10·11. $C_{32}H_{50}O_4$ requires: C, 79·66; H, 10·37%).

Preparation of aleuritolonic acid from III. Methyl aleuritolonate (0·15 gm) was hydrolysed by the same method described above.[7] The product after crystallization from $CHCl_3$–MeOH mixture gave crystalline aleuritolonic acid, m.p. 280–82°. (Found: C, 79·20; H, 10·30. $C_{30}H_{46}O_3$ requires: C, 79·29; H, 10·18%).

Isomerization of acetyl methyl aleuritolate and preparation of acetyl methyl oleanolate. Compound I (0·20 gm) was isomerized by heating for 15 min with conc HCl and AcOH. The crystalline solid obtained after usual work up on crystallization from $CHCl_3$–MeOH mixture afforded pure acetyl methyl oleanolate (0·17 gm), m.p. 219–20°, $(\alpha)_D$ + 58·82° identical with an authentic sample (m.m.p. and IR). (Found: C, 76·86; H, 9·83. Calc for $C_{33}H_{52}O_4$; C, 77·34; H, 10·15%).

Perbenzoic acid titration of acetyl methyl aleuritolate I. Compound I (0·0548 gm) was titrated with 1·22 N perbenzoic acid in $CHCl_3$. It took up one mole equivalent of perbenzoic acid within 24 hr with no further uptake indicating the presence of one double bond.

LAH reduction of methyl aleuritolate (II). To a soln of II (0·15 gm) in dry dioxan (15 ml) was added LAH (0·075 gm) and the mixture was heated on a water bath for 4 hr. After the reaction, excess LAH was destroyed by careful addition of moist ether and then with a satd Na_2SO_4aq. The ethereal soln was washed with water and dried (Na_2SO_4). On removal of the solvent, a solid was obtained which after crystallization from $CHCl_3$–MeOH gave pure crystals of V, m.p. 265–7°, identical with an authentic sample (m.m.p. and IR). (Found: 81·23; H, 10·51. Calc for $C_{30}H_{50}O_2$; C, 81·39; H, 11·38%).

Myricadiol diacetate VI. Acetylation of V with Ac_2O-pyridine in the usual manner afforded pure crystalline VI, m.p. 251–52°, $(\alpha)_D$ −3°, identical with an authentic sample. (Found: C, 78·04; H, 9·89. Calc for $C_{34}H_{54}O_4$: C, 77·56; H, 10·26%).

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