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TRITERPENOID FROM BETULA UTILIS— ISOLATION AND STRUCTURE OF KARACHIC ACID†

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Abstract. Karachic Acid—a substance isolated from the bark of *Betula utilis*—has been shown to be 3 β-hydroxy-6α-acetoxyoleanoic acid—a new triterpenoid of the oleanoic acid family.

Betula utilis is a tree commonly found at high altitudes in the temperate Himalayas extending from Chitral eastwards to Azad Kashmir and in Sikkim and Bhutan. The infusion of its bark has found wide use in indigenous medicine as an antiseptic, carminative and in hysteria.[1] The outer bark of the plant has been reported to contain the triterpenoids betulin, lupeol, oleanolic acid, acetyl oleanolic acid and leucocyanidin.[2]

Our interest in the systematic investigation of the constituents of Pakistani plants, particularly those used in indigenous medicine[3,4] led us to a chemical investigation of the constituents of the bark of *Betula utilis*. The ethereal extracts of the ground bark afforded betulin. After betulin had been completely removed by repeated concentration and crystallization of the ethereal extracts, the ethereal solution was evapporated and the petroleum ether extracts of the residue concentrated to afford a colourless crystalline solid, —Karachic acid, m.p. 260°—261°, $[\alpha]_D^+$ 79 (Pyridine), +83 ($CHCl_3$). Karachic acid did not correspond with any of the substances earlier reported from the bark of this plant[2] in its physical characteristics.

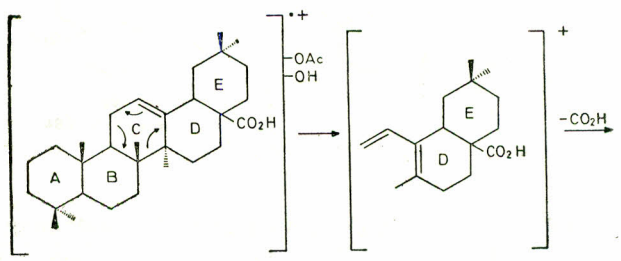
Microanalysis indicated the molecular formula to be $C_{32}H_{48}O_5$. The IR spectrum showed absorptions at 1730 and 1680 cm^{-1} assigned to ester and carboxyl absorptions respectively. A broad absorption at 3100–3300 cm^{-1} indicated the presence of one or more hydroxyl groups. The UV spectrum showed end-absorption only.

Karachic acid afforded a monoacetyl derivative, m.p. 315°, on treatment with acetic anhydride and pyridine With benzoyl chloride and pyridine, a monobenzoyl, derivative was obtained, m.p. 218–220°. These experiments suggested that only one hydroxyl group was present

The NMR spectrum of Karachic acid in $CDCl_3$ showed 6 three-proton singlets at 0.64, 0.81, 0.92, 0.98, 1.00 and 1.18δ, assigned to the seven quaternary methyls at C-26, C-24, C-29/30, C-29/30, C-25, C-23 and C-27 respectively. A three-proton singlet at 2.07δ showed that the ester function was in the form of an acetyl rather than a carbomethoxy group which would have resonated farther downfield. The proton *geminal* to the hydroxyl group resonated as a one-proton multiplet at 2.75–3.0δ. Another one-proton multiplet was observed in the olefinic region at 5.3δ. A third one-proton multiplet appeared at 4.53δ. This was assigned to the proton *geminal* to the hydroxyl group in accordance with the positions of similar protons in other acetylated triterpenes.[5–7]

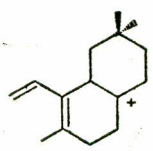
Mass spectrometry provided extremely valuable information about the structure of the substance under investigation. The molecular ion was found to occur at *m/e* 572 and high resolution mass spectrometry showed the exact mass of this peak to be 512.3437, in confirmation of the molecular formula $C_{32}H_{48}O_5$ assigned to Karachic acid. The base peak occurred at *m/e* 248. This immediately established that the substance was a pentacyclic triterpene of the β-amyrin series with a 12,13 double bond. Such compounds readily undergo a characteristic retero Diels–Alder type fragmentation of ring C to provide a major peak for the ion (II) containing rings D and E.[8]

The fragmentation indicated that the hydroxyl and acetoxyl groups were located in rings A and/or B and not in rings, C,D or E as a fragment of *m/e* 248



I II

m/e 248.1778



III

m/e 203.1794

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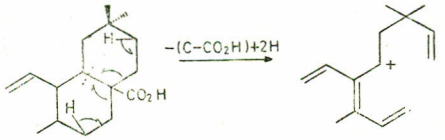
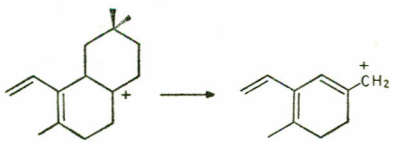
would not then have been obtained. The carboxyl group on the other hand was present in this fragment and the ready loss of this group from fragment (II) was in agreement with its positioning at C-17, a common cleavage in other members of the oleanoic acid family. This cleavage was confirmed by the presence of a distinct metastable peak at 166.1. Another fragment at 189 *m/e* was also formed by fragmentation of (II) probably involving the loss of C-17 alongwith the CO_2H group with a double hydrogen transfer to afford the conjugated allylic cation (II) (metastable at *m/e* 144).



II
m/e 248.1778

IV
m/e 189.1648

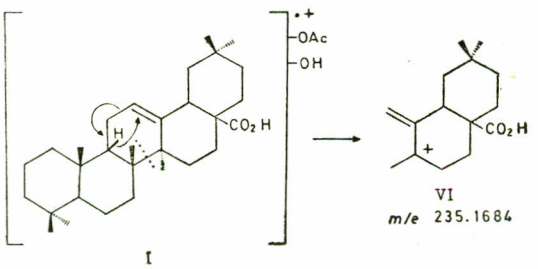
Another fragment at *m/e* 133, caused by the rupture of ring E from the fragment (III), was also discernible. This was confirmed by the presence of a metastable peak at 87.1.



III
m/e 203.1792

V
m/e 133.1015

A peak at *m/e* 235 appeared to be due to the fragmentation of ring C as shown below:

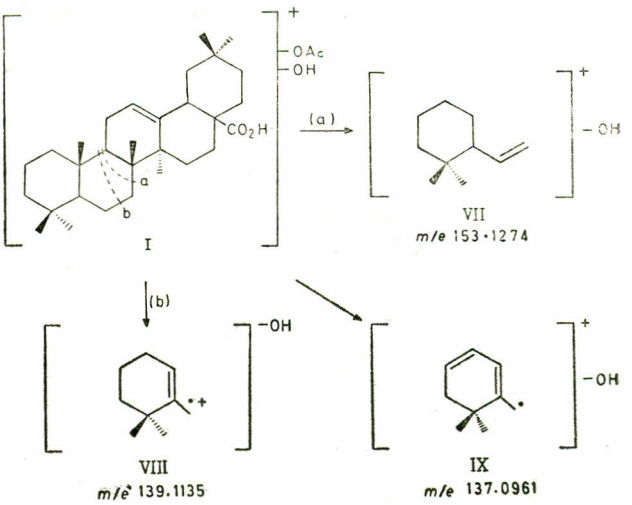


I

VI
m/e 235.1684

Other fragments (VII, VIII and IX) containing a single oxygen were discernible at *m/e* 153. 1274 ($C_{10}H_{17}O$), 139.1135 ($C_9H_{15}O$) and 137.0961 ($C_9H_{13}O$) respectively. These appeared to be ring A containing fragments formed by cleavage across ring B. It is interesting that these fragments contained the hydroxyl group, suggesting that the acetoxyl group was located in ring B.

That the hydroxyl and acetoxyl groups were located separately in rings A and B was also indicated by the NMR spectrum. Thus a vicinal disposition of the two groups was ruled out as hydrolysis of Karachic acid afforded the corresponding crystalline diol, m.p. 280°, which failed to form an acetonide. Moreover, the coupling pattern of the protons *geminal* to the hydroxyl and acetoxyl groups was not consistent with their vicinal disposition. A 1:3 disposition of the two hydroxyl groups in the diol was also not possible as oxidation with chromic acid would then have afforded a 1:3 dione, readily recognizable by the characteristic



I

VII
m/e 153·1274

VIII
m/e 139.1135

IX
m/e 137.0961

UV spectrum of the corresponding β-ketoenolate under basic conditions. No such dione was formed on oxidation. In view of this evidence and taking into consideration the chemical shift of the proton *geminal* to the hydroxyl group, it is clear that the hydroxyl must be located at C-3 in a β-disposition.

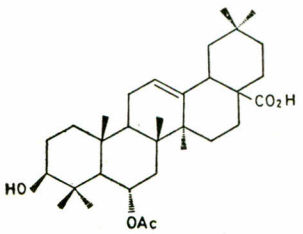
Oxidation of the diol from Karachic acid with Jones reagent resulted in the formation of a ketonic compound. The mass spectrum of the substance showed the molecular ion at *m/e* 468 indicating that only one of the hydroxyl groups of the diol had been oxidized under the mild conditions used. Tertiary nature of this inert hydroxyl was ruled out on grounds of the presence of the *geminal* downfield proton, and its resistance to oxidation indicated that this hydroxyl was derived from the acetoxyl which must be located at a hindered position in ring B, since hydroxyl groups in ring A at positions 1,2 and 3 are known to undergo oxidation readily. This showed that the acetoxyl was located at C-6 in ring B, this position being hindered by the 24, 25 and 26 methyls. The orientation of this acetoxyl must be equatorial because in a β- disposition the 24,25 and 26 methyl groups would have been shifted significantly downfield by the 1,3-diaxial interactions.

Further evidence for the fact that it was the hydroxyl and not the acetoxyl group which was located at C-3 was provided by the NMR spectrum. It has been shown by Tursch and his coworkers[5] that when a —OH was located at C-3, the 23 methyl resonated at about 1.00δ whereas when an acetoxyl group was located at this carbon, then the 23 methyl appeared farther upfield at 0.86δ. In Karachic acid the 23 methyl appeared at 1.00δ, thus confirming the location of the hydroxyl group in ring A.

An alternative position of the —COOH group which would have afforded similar fragments in the mass spectrum is in place of the 29-methyl. In such substances, however, the 30-methyl would be expected

to appear downfield at about 1.23δ[5]. The 29 and 30 methyls were, however, found to resonate at 0.9δ and 0.96δ, this being the normal position for such methyls in oleanoic acid derivatives possessing a C-28—COOH group.

In the light of the above evidence Karachic acid is suggested to be olean-3β-hydroxy-6α-acetoxy-12-enoic acid (I).



Karachic acid (I)

Experimental

Well-ground *Betula utilis* bark (100g) was extracted with about 2 liters ether for 12 hr in a Soxhlet apparatus. The ethereal extract on cooling deposited 11.25 g of crude betulin, m.p. 250–52°, which was filtered out. It did not give any depression in melting point on admixture with an authentic sample of betulin. The filtrate was concentrated and on the addition of petroleum ether (b.p. 60–80°) some more betulin separated which was again filtered out. This procedure was repeated three times, when almost all of the betulin (10.18 g) was removed from the filtrate making up a total of 21.43 g of betulin. It was then freed of the solvent and thus a petroleum-ether soluble fraction (2.3 g) was obtained which on crystallisation from alcohol gave silky needles of *karachic acid.* It was recrystallised five times from alcohol when it finally melted at 260–61°C, $[\alpha]_D^{25}$ +79° (pyridine) and + 83° ($CHCl_3$). It analysed for $C_{32}H_{48}O_5$. Found after drying to constant weight at 100° *in vacuo*: C, 75.9; H, 9.95. Calculated. for $C_{32}H_{48}O_5$: C, 75.0; H, 9.4% IR (nujol), 3400 cm^{-1} (—OH, broad) 1735 cm^{-1} (—$OCOCH_3$), 1690 cm^{-1} (—COOH); NMR ($CDCl_3$): 0.64δ, (s, 3H methyl), 0.81δ (s, 3H, 24 methyl) 0.92 (s, 3H, 29/30 methyl). 0.96δ (s,3H, 29/30 methyl), 0.98δ(s,3H, 25 methyl), 1.00δ (s, 3H, 25 methyl), 1.18δ (s, 3H, 27 methyl), 2.07δ (s, 3H, —$OCOCH_3$), 2.9δ (m, IH, C—3H), 4.53δ (m, IH, C—7H), 5.3δ (m, IH, olefinic H), mass spectrum M^+ 512.3437 (—calc. for $C_{32}H_{48}O_5$:512.3501), major peaks at 248 (100%), 203, 190, 189; high resolution mass spectrum of important peaks: 248.1778 (calc. for $C_{16}H_{24}O_2$; 248.1778), 203.1794 (calc. for $C_{15}H_{23}$, 203.1799), 189.1648 (calc. for $C_{14}H_{21}$, 189.1643), 235. 1685 (calc. for $C_{15}H_{23}O_2$; 235.1697), 153.1274, (calc. for $C_{10}H_{17}O$; 153.1279), 139.1135 (calc. for $C_9H_{15}O$; 139.112), 137.0961 (calc. for $C_9H_{13}O$;137.0966), 133.1015 (calc. for $C_{10}H_{13}$; 133.1017); UV. spectrum: end absorption only.

Hydrolysis of Karachic Acid. Karachic acid (20 mg, 0.04 mmole) was dissolved in 5 ml of a 10% solution of NaOH in $MeOH/H_2O$ (1:1) and stirred for 12 hr at room temperature. The solution was evaporated to dryness and the residue partitioned between water and ethyl acetate (10 ml). The ethyl acetate layer was separated, dried (Na_2SO_4), and evaporated to afford a white crystalline solid. Recrystallisation from ethyl acetate afforded colourless crystals of the diol, m.p.280°. Found: C,76.4; H,9.8% Calc. for $C_{30}H_{46}O_4$: C, 76.6; H, 9.7%); IR (nujol), 1690 cm^{-1} (—COOH), 3400 cm^{-1} (broad—OH), no ester absorption, mass spectrum: 470 (4%, M^+), 456 (5%), 454 (7%), 249 (19 %), 248 (100%), 233 (9%), 207 (16%), 203 (45%), 190 (9%), 189 (10%), 175 (8%), 133 (11%) 121 (8%), 119 (12%), 95 (17%), 81 (17%), 69 (25%), 55 (22%).

Oxidation of Diol. The diol (10 mg, 0.021 mmole) was dissolved in 1 ml acetone and chromic acid (0.017 g), [standard solution prepared by dissolving CrO_3] (2.6 g) in conc. H_2SO_4 (2.3 mol) and diluting with H_2O to 10 ml] was added. The solution was stirred for 15 min at 25°, poured into 50 ml cold water and extracted with 3 × 5 ml portions of ether. The extracts were dried, filtered and evaporated to afford a colourless crystalline solid (8.0 mg) which was examined by mass spectroscopy. Mass spectrum 468 (M^+, 2%) 454 (4%), 439 (1%), 428 (4%) 393 (2%) 249 (6%), 248 (27%), 213 (8%), 203 (21%), 170 (63%), 141 (5%), 77 (100%).

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