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KARACHIC ACID: A NEW TRITERPENOID FROM *BETULA UTILIS*

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Key Word Index—*Betula utilis*; Betulaceae; bark; structure elucidation; pentacyclic triterpenoids.

Abstract—Karachic acid, from the bark of *Betula utilis*, has been shown to be 3β-hydroxy 6α-acetoxy oleanoic acid, a new triterpenoid of the oleanoic acid type.

Betula utilis grows in the temperate Himalayas from Kashmir in Pakistan to Sikkim and Bhutan. The infusion of its bark has been used in indigenous medicine as an antiseptic, carminative and in hysteria [1]. Seshadri and coworkers have previously reported that the outer bark contains the triterpenoids butulin, lupeol, oleanolic acid, acetyl-oleanolic acid and leucocyanidin [2].

Our continuing interest [3, 4] in the constituents of Pakistani plants led us to a chemical investigation of *Betula utilis* bark. From the ether extracts of the ground bark, betulin was obtained. On complete removal of betulin by repeated concentration and crystallization of the ethereal extracts, the ethereal solution was evaporated and the residue taken up in petrol (bp 60–80°) and the petrol soluble portion afforded a colourless crystalline solid which on repeated crystallization from alcohol gave karachic acid $C_{32}H_{48}O_5$, mp 260–261°, $[\alpha]_D$ +79° (pyridine), $[\alpha]_D$ +83° ($CHCl_3$). Karachic acid did not correspond with any of the materials reported earlier by Seshadri [2].

The IR spectrum revealed absorptions at 1730 cm^{-1} and 1680 cm^{-1} assigned to ester and carboxyl groups respectively. A broad absorption in the region of 3100–3300 cm^{-1} was indicative of the presence of a hydroxyl group (s). The UV spectrum showed end absorption only. Karachic acid afforded a monoacetate mp 315° and a monobenzoate mp 218–220°, indicating that it contained a single hydroxyl group.

The NMR spectrum of karachic acid in $CDCl_3$ showed six sharp three-proton singlets at 0·64 δ, 0·81 δ, 0·92 δ, 0·98 δ and 1·18 δ 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. Another three-proton singlet was located at 2·07 δ, indicating that the ester function was in the form of an acetyl rather than a carbomethoxy group. A one-proton multiplet was visible at 2·75–3·0 δ which was assigned to the proton alpha to the hydroxyl function. Another one-proton multiplet was observed at 5·3 δ due to the olefinic proton. A third multiplet centred at 4·53 δ, also integrating for one proton was ascribed to the proton alpha to the acetoxyl function in agreement with the known values for similar acetylated triterpenes.

The MS showed the molecular ion at m/e = 512 and high resolution mass spectrometry demonstrated the exact mass of this peak to be 512·3437, in agreement with the molecular formula $C_{32}H_{48}O_5$. The base peak occurred at m/e = 248. This immediately showed that the substance was a pentacyclic triterpene of the β-amyrin series with a 12–13 double bond [8].

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

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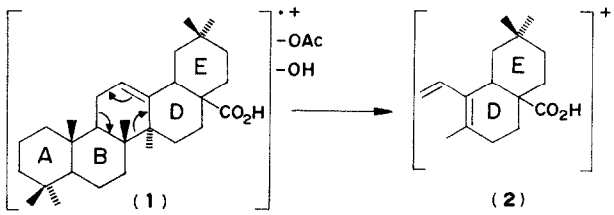
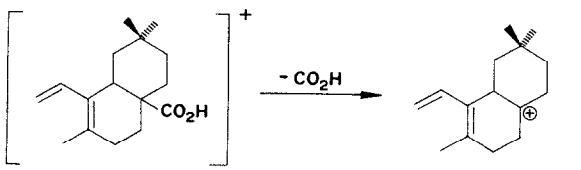


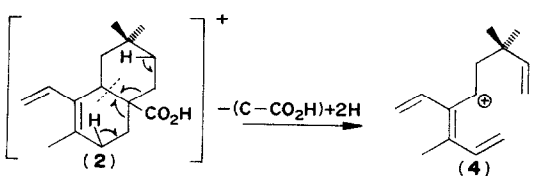
(1) m/e = 512·3437 (2) m/e = 248·1778

would not then have been obtained. The carboxyl group was also present in this fragment and the ready loss of this group from the fragment (**2**) to afford the fragment (**3**) was in agreement with its positioning at C-17.



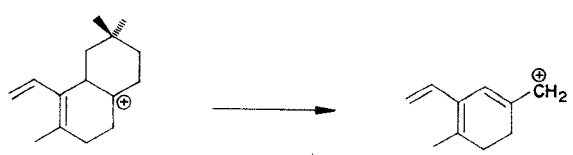
(2) m/e = 248·1778 (3) m/e = 203·1794

This cleavage was confirmed by the presence of a distinct metastable peak at 166·1. Another fragment at 189 m.u. was also formed by fragmentation of (**2**) probably involving the loss of C-17 along with the CO_2H group with a double hydrogen transfer to afford the conjugated allylic cation (**4**) (metastable at m/e = 144).



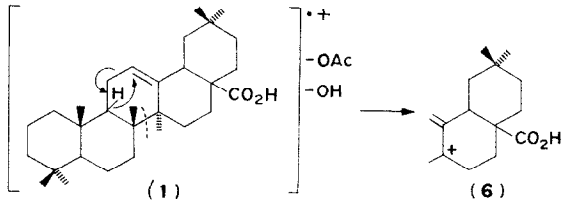
(2) m/e = 248·1778 (4) m/e = 189·1648

Another fragment at m/e = 133, caused by the rupture of ring E from the fragment (**3**) was also discernible (metastable peak at 87·1).



(3) m/e = 203·1792 (5) m/e = 133·1015

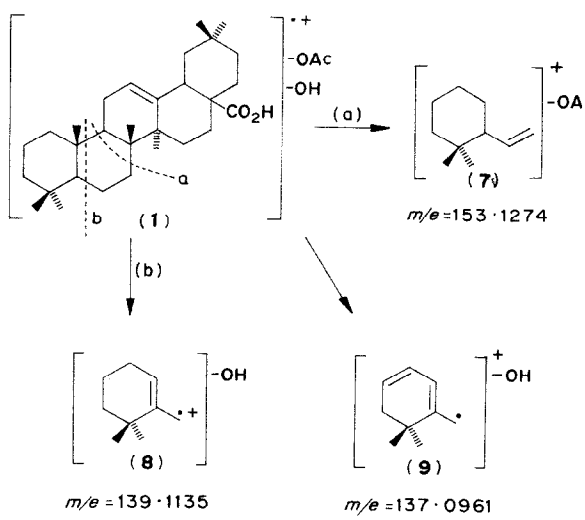
A peak at m/e = 235 appeared to be due to the fragmentation of ring C as shown:



(1) (6) m/e = 235·1684

Other fragments **7**, **8** and **9** 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.

A vicinal disposition of the two groups was ruled out since hydrolysis of karachic acid afforded the corresponding crystalline diol mp 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 since oxidation with chromic acid would then have afforded a 1:3 dione, readily recognizable by the characteristic UV spectrum of the corresponding β-ketoenolate under basic conditions: no such dione was formed on oxidation.

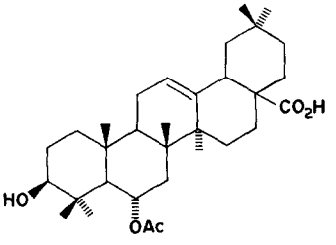


In view of the above evidence taking into consideration both the chemical shift of the proton geminal to the hydroxyl group as well as the pre-

ferred biogenetic location of the hydroxyl group in such systems, it is suggested that the hydroxyl is located at C-3 in a β disposition.

Oxidation of the diol from karachic acid with Jones reagent resulted in the formation of a ketone, the MS of which showed the molecular ion at $m/e = 468$ indicating that only one of the hydroxyl groups of the diol had been oxidized. The 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 it 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 suggested 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.

It has been shown by Tursch and co-workers [5] that when a β-OH is located at C-3 in triterpenoids, the 23 methyl resonates at about 1·00 δ whereas when an acetoxyl group is located at this carbon, then the 23 methyl appears farther upfield at 0·86 δ. In karachic acid the 23 methyl appeared at 1·00 δ, 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 1·23 δ [5]. The 29- and 30-methyls were, however, found to resonate at 0·9 and 0·96 δ, this being the normal position for such methyls in oleanoic acid derivatives possessing a C-29—COOH group. In the light of the above evidence, karachic acid is suggested to be olean 3-β hydroxy-6α acetoxy-12-enoic acid (**1**).



(**1**)

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