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STUDIES ON THE CHEMICAL CONSTITUENTS OF AZADIRACHTA INDICA A.JUSS (MELIACEAE) PART I: ISOLATION AND STRUCTURE OF A NEW TETRANORTRITERPENOID — NIMOLICINOL

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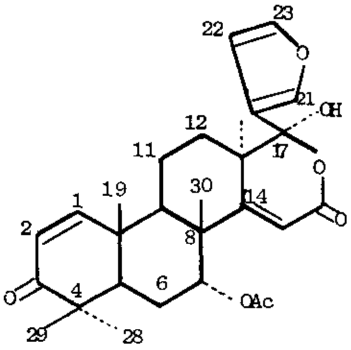
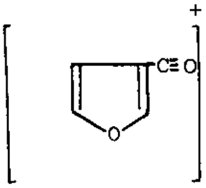
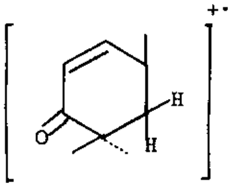
Abstract- A new tetranortriterpenoid provisionally named as nimolicinol has been isolated from the fresh, ripe, undried fruits of *Azadirachta indica* A. Juss (Neem) and its structure has been elucidated as 17α-hydroxy-14,15-deoxy-17-epi-gedunin (I) by spectral studies.

In view of the enormous therapeutic importance[1,2] attributed to *Azadirachta indica*, different parts of the tree have been subjected to chemical investigations by various groups of workers.[3-8] More recent studies undertaken for the first time on fresh, undried fruits of the plant by Siddiqui, et al. led to the isolation and structure elucidation of three new tetranortriterpenoids, azadiradione,[9] epoxyazadiradione,[10] and 17α-hydroxyazadiradione.[11] The present communication deals with the isolation of a new bitter constituent nimolicinol from the Neem fruit, and the elucidation of its structure as 17α-hydroxy-14,15-deoxy-17-epi-gedunin (I), on the evidence of spectral data. It is noteworthy in this connection that throughout the isolation procedure, mild conditions of acid/base and temperature were employed to avoid the possible formation of artifacts.

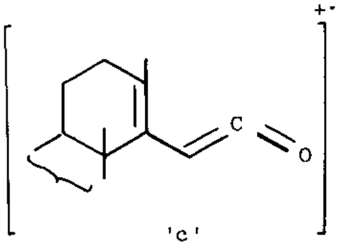




'a'



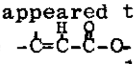
'b'



'c'

The mass spectrum of nimolicinol showed a molecular ion peak at m/e 482.2293 corresponding to the formula $C_{28}H_{34}O_7$ (calculated 482.2304). The ir spectrum revealed bands at 1665 cm^{-1} (α,β-unsaturated carbonyl), 1710 cm^{-1} (carbonyl function of α,β-unsaturated δ-lactone), 1740 cm^{-1} (ester carbonyl), 3460 cm^{-1} (O-H) and 875, 1502, 3150 cm^{-1} (characteristic of a β-substituted furan ring). The uv spectrum showed maxima at 218 nm (log ε 4.28) and 231 nm (log ε 4.47) which are in keeping with a furan ring and 1-en-3-one functions.

The molecular formula revealed 12 double bond equivalents in the molecule, 3 of which have been accounted for by a furan ring, 3 by three carbonyl functions, 4 by four rings while two must be present as double bonds since the ^{1}H-nmr spectrum showed three one-proton olefinic signals at δ 7.05 (d, J = 10 Hz, H-1), δ 5.85 (d, J = 10 Hz, H-2) and δ 5.57 (s, H-15). Three one-proton multiplets at δ 7.52, δ 7.40 and δ 6.45 have been assigned to H-21, H-23 and H-22 respectively and a one-proton multiplet at δ 5.20 ($W_{h/2}$= 7.5 Hz) has been attributed to H-7. The width of this multiplet at half height corresponded to an equatorial proton, suggesting that orientation of the acetoxy function is α. A one-proton singlet at δ 4.31 which disappeared on shaking with D_2O has been ascribed to the hydroxyl proton. A three-protons singlet of the acetyl protons appeared at δ 1.95, while four singlets for five tertiary methyl groups appeared at δ 1.06 (6H), δ 1.12, δ 1.22 and δ 1.35.

The data presented so far suggested the presence of a furan ring and normal A and B rings of gedunin[12] containing 7α-acetoxy function. The observation, that the hydroxyl function could not be acetylated with pyridine and acetic anhydride under normal conditions, led to the assumption that it is tertiary in nature and located at one of the three positions, i.e. C-5, C-9 or C-17. The absence of characteristic H-17 signal[12] in the ^{1}H-nmr spectrum, however, revealed that the hydroxyl group is at C-17, which was further corroborated from ^{13}C-nmr (Table) and mass spectral data. Appearance of only one olefinic proton at δ 5.57 (apart from H-1, H-2 and furanoid protons) implied that the double bond had to be placed in a way which would produce such a single proton, i.e. either at C-9(11) or C-14(15). As the ir spectrum and the double bond equivalents were in agreement with an α,β-unsaturated lactone, it appeared to indicate that this olefinic proton is a part of the partial structure  $-\overset{|}{C}=\overset{H}{C}-\overset{O}{\overset{\|}{C}}-O-$. Thus the possible positions for the lactone were either in ring C or D. The ^{13}C-nmr and mass spectral data, however, conclusively supported ring D lactone, as C-17 appeared at δ 104.0 revealing that it is bonded to two oxygen functions.

The chemical shifts at δ 1.06 (6H) and δ 1.22 (3H) have been assigned to 4α-, *4β-CH_3 protons and 10-CH_3 protons respectively through comparison of shifts for these* protons in gedunin[13] and deoxygedunin.[12] The downfield shift at δ 1.35 (3H) has been attributed to 8-CH_3 protons in analogy to its value observed in deoxygedunin in which these protons appear at downfield due to an anisotropic effect of the 14-15 double bond,[14] whereas the remaining band at δ 1.12 has been ascribed to 13-CH_3 protons.

The configuration of furan ring in nimolicinol has been determined by measuring nuclear Overhauser effects (NOE). Irradiation at δ 1.35 (H-30) enhanced the integral intensities of H-7 at δ 5.20 (18% NOE), H-21 at δ 7.52 (24% NOE) and H-15 at δ 5.57 (8.3% NOE). Irradiation at δ 1.12 (H-18) increased the signal of H-9 at δ 2.42 (16% NOE), whereas the signals at δ 7.52 (H-21) and δ 6.45 (H-22) remained unaffected. The furan ring is therefore β-oriented like in 17-epi-azadiradione.[8]

The chemical shifts of C-18 and C-20 in the ^{13}C-nmr spectrum also supported this view. The upfield appearance of C-18 in nimolicinol as compared to its downfield shift in 17β-hydroxyazadiradione[8] is due to the γ-gauche interaction of this methyl group with the 17-hydroxyl function which provided a definite evidence that the hydroxyl function is α-oriented. Further, the chemical shift of C-20 in nimolicinol is also in agreement with the α-orientation of the hydroxyl function.[8,11,15]

The fragments at m/e 95.0120 and m/e 137.0964 in the mass spectrum corresponding to the compositions $C_5H_3O_2$ and $C_9H_{13}O$ may be attributed to the ions 'a' and 'b' respectively. Another fragment at m/e 370.2121 which is equivalent to $C_{23}H_{30}O_4$ (ion 'c') is consistent with retro Diels-Alder cleavage of ring D, whereas the base peak at m/e 328.2035 ($C_{21}H_{28}O_3$) possibly results from the loss of a ketene molecule from the ion 'c'. Fragments observed at m/e 465, 467 and 422 have been attributed to the loss of OH, CH_3 and CH_3COOH respectively.

TABLE

^{13}C-NMR SPECTRAL DATA

Carbon Nos.	δ	Carbon Nos.	δ	Carbon Nos.	δ	Carbon Nos.	δ
1	156.9d	8	43.9[a]s	15	111.1[b]d	22	110.1[b]d
2	125.1d	9	45.5d	16	164.0s	23	141.8d
3	204.0s	10	42.1[a]s	17	104.0s	28	20.9[d]q
4	43.1[a]s	11	15.2t	18	23.2[c]q	29	26.9q
5	40.5d	12	37.2t	19	18.9q	30	22.9[c]q
6	25.0t	13	44.5s	20	125.8s	$-O-\overset{O}{\overset{\|}{\underline{C}}}-CH_3$	169.8s
7	73.4d	14	170.9s	21	142.7d	$-O-\overset{O}{\overset{\|}{C}}-\underline{CH_3}$	21.1[d]q

a,b,c,d: Assignments may be reversed. All values are in δ (ppm) relative to TMS=0.

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. Ir (KBr disc) and uv spectra (in methanol) were measured on JASCO IRA-I spectrometer and Pye-Unicam SP-800 spectrometer respectively; mass spectra were recorded on Finnigan MAT 312 double focussing mass spectrometer. ^{1}H- and ^{13}C-nmr (broad band and off-resonance) spectra were recorded in $CDCl_3$ on a Bruker WP-100-SY FT-NMR spectrometer. ^{13}C-nmr spectral assignments have been made partly through the multiplicities in the off-resonance decoupled spectrum and partly through comparison with published data for similar compounds.[8-11,16] The purity of samples was checked on tlc (silica gel).

Fresh, unruptured, ripe fruits of Neem (20 Kg) were repeatedly percolated with ethanol. The thickish residue, obtained on removal of the solvent in vacuo, was partitioned between ethyl acetate and water. The ethyl acetate layer was repeatedly extracted out with 1% NaOH to separate the acidic part from the neutral constituents. The ethyl acetate layer was washed with water, dried and filtered. The darkish residue left on removal of the solvent under reduced pressure was taken up in 50% ethanol, and repeatedly shaken out with a mixture of benzene and petroleum ether (2:1). The benzene-petroleum ether layer was charcoaled, freed of the solvent in vacuo and divided into petroleum ether soluble and insoluble fractions. On keeping at room temperature the concentrated solution of the former yielded a fine crystalline mass which on recrystallization from ethyl acetate, could be identified as azadiradione (loc.cit) through comparison of its spectral data (uv, ir, mass, ^{1}H-nmr), mp, mmp, tlc and mixed tlc with an authentic sample. The mother liquor of azadiradione and the petroleum ether insoluble fraction were combined together, taken in ethyl acetate and shaken out with 6% sodium carbonate solution to remove the residual acidic components. After usual working, the ethyl acetate layer was charcoaled, concentrated and kept at room temperature overnight, when fine crystals of nimolicinol (1 g, 0.01% on dry weight basis of fruits) were obtained. The mother liquor yielded, on concentration another crop of nimolicinol. On recrystallization from ethyl acetate-methanol (1:1) it formed fine shining needles, showing one uv active spot on tlc, mp 270-274°C.

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