ISOAZADIROLIDE, A NEW TETRANORTRITERPENOID FROM AZADIRACHTA INDICA A.JUSS (MELIACEAE)

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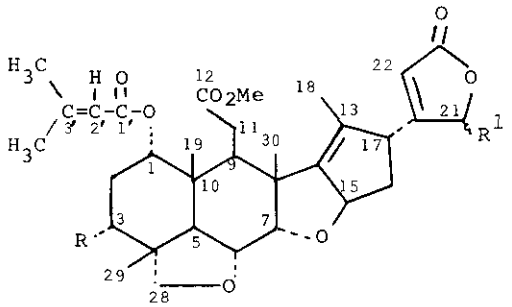
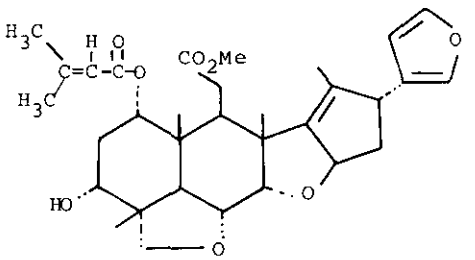
Abstract - A new ring-C seco tetranortriterpenoid γ-hydroxybutenolide named as isoazadirolide, has been isolated from the acidic fraction of the fresh, undried, winter leaves of Azadirachta indica A.Juss (neem), along with a coumarin identified as scopoletin. The structures of these compounds have been established through chemical and spectral studies. It is the first instance of isolation of a coumarin from any of the various parts of neem tree.

Chemical investigations undertaken by Siddiqui et al. in the terpenoidal constituents of Azadirachta indica have led to the isolation and structure elucidation of several new triterpenoids from the fresh fruits[1-5] and leaves[5-9]. In continuation of these studies, a new γ-hydroxybutenolide tetranortriterpenoid named as isoazadirolide has been isolated from the acidic fraction of the fresh, undried neem leaves, together with a coumarin identified as scopoletin through the comparison of its spectral data with those reported in literature[10,11]. The structure of isoazadirolide has been established as I, through chemical and spectral studies.

Isoazadirolide (I) has molecular formula $C_{32}H_{42}O_{10}$ (high resolution mass) and showed uv absorption maxima at 210 (ε41020) and 290 nm (ε 3516). The ir spectrum showed the presence of hydroxy group (3400 cm^{-1}), α,β-unsaturated-γ-lactone (1760 cm^{-1}), carbomethoxyl function (1740 cm^{-1}), ester carbonyl (1720 cm^{-1}), trisubstituted double bond (1640 and 822 cm^{-1}) and ether linkage (1145 and 1080 cm^{-1}). The ^{1}H-nmr spectrum of I showed two one-proton multiplets at δ 5.06



I: R = R^1 = OH
II: R = R^1 = OAc



2',3'-dehydrosalannol

($W_{\frac{1}{2}}$ = 5.3 Hz) and 4.91 ($W_{\frac{1}{2}}$ = 5.7 Hz) related to H-1 and H-3 respectively; five one-proton multiplets at δ 5.50, 2.32, 2.22, 2.10 and 2.06 due to H-15, H-11β, H-2β, H-16β and H-2α respectively; five one-proton doublets at δ 4.21 (J = 3.2 Hz), 4.08 (J = 7.3 Hz), 3.72 (J = 9.0 Hz), 3.61 (J = 7.3 Hz) and 2.60 (J = 12.3 Hz) attributable to H-7, H-28α, H-17, H-28β and H-5 respectively. Four one-proton double doublets at δ 3.97 (J = 12.3 and 3.2 Hz), 2.52 (J = 5.7 and 5.5 Hz), 2.30 (J = 13.0 and 7.0 Hz) and 2.20 (J = 16.0 and 5.7 Hz) have been assigned to H-6, H-9, H-16α and H-11α respectively. It further showed a two-proton broad multiplet at δ 3.84 for the hydroxyl groups; a three-proton sharp singlet at δ 3.50 for methoxy protons; a three-protons broad singlet at δ 1.68 for a vinylic methyl, and three three-proton sharp singlets at δ 1.13 (H-19), 1.24 (H-29) and 1.28 (H-30) for three quaternary methyl groups. The chemical shifts of the protons of carbocyclic nucleus are in agreement with those reported for the same protons in salannol[12] and 2',3'-dehydrosalannol[13]. The ^{1}H-nmr spectrum further showed a senecioxy ester function by the presence of an olefinic proton singlet at δ 5.92 (H-2') and two vinylic methyl groups at δ 2.14 and 1.94 ($\rangle$C=CHCO). These values are in keeping with those reported for the same protons in 2',3'-dehydrosalannol (loc.cit.). The presence of this group was further corroborated by a diagnostic fragment at m/z 486.2266 ($C_{27}H_{34}O_8$) in the mass spectrum of **I**, resulting from the loss of senecioic acid. Further, the signals characteristic of the furan ring were missing and a 21-hydroxybut-20(22)-ene-21,23-γ-lactone side chain was indicated by the presence of two one-proton multiplets at δ 6.00 (H-21) and 6.15 (H-22) in the ^{1}H-nmr spectrum of **I**. On acetylation with acetic anhydride-pyridine, **I** yielded the diacetyl derivative **II**, in the ^{1}H-nmr of which (vide experimental) the signals corresponding to the protons geminal to the hydroxyl function shifted to δ 5.44 and 6.89, along with the appearence of two acetoxy methyl signals at δ 1.97 and 1.98. The data recorded so far left the location of the senecioxy ester and one of the hydroxyl functions which could be placed in ring-A at C-1 and C-3, since the multiplets of H-1 and H-3 have similar widths at half height. Further, the multiplicity and value of $W_{\frac{1}{2}}$ (5.3 and 5.7 Hz respectively) of these protons showed that they are equatorial and coupled with two neighbouring protons i.e. H-2α and H-2β. The placement of the ester function at C-1 and the hydroxyl group at C-3 could be finally established through comparison of the chemical shifts of various protons with those of 3-deacetylsalannin and salannol (loc.cit.), particularly of H-9, H-11α and H-11β; and H-5, H-28α and H-28β.

The stereochemistry of various centres of isoazadirolide (**I**) has been established through NOESY spectrum which showed the spatial connectivities of H-17 with H-30, H-16β and H-7; H-30 with H-18, H-16β, H-11β, H-7, H-6 and H-1; H-19 with H-30, H-6 and H-2β; H-29 with H-28β,

and H-7; H-5 with H-28α and H-15; and also of H-6 with H-7. The spatial connectivity of H-17 with H-30, H-16β and H-7 indicated that the side chain at C-17 is α oriented. The spatial connectivity of H-30 with H-1 further confirmed the α orientation of the ester substituent at C-1.

Isoazadirolide (**I**) is of significant biological importance as other γ-hydroxybutenolides have been reported to possess insect growth regulating[5] and insect antifeeding[14] properties. Moreover, it has also been observed earlier that many of the most potent insect feeding deterrent limonoids are ring-C seco type[15]. Further, as the hydroxybutenolide side chain has been considered as the intermediate in the formation of furan ring of meliacins[16], **I** may be regarded as the precursor of salannol and 2',3'-dehydrosalannol isolated earlier from neem (loc.cit.).

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. Ir and uv spectra were measured on JASCO IRA-I and Pye-Unicam SP-800 spectrometers respectively. Mass spectra were recorded on Finnigan MAT 112 and 312 double focussing mass spectrometers; exact masses have been measured through peak matching. ^{1}H-nmr spectrum and NOESY experiment (pulse delay 2 sec., mixing time 0.5 sec.) were run on Bruker AM 300 NMR spectrometer. Merck Kieselgel 60 PF_{254} coated on glass plates was used for analytical (thin layer) and preparative (thick layer) chromatography.

Isolation of isoazadirolide (I)

Winter crop of fresh, undried and uncrushed neem leaves (40 kg) collected from Karachi region, were repeatedly percolated with ethyl alcohol at room temperature. Removal of the solvent from the combined extracts under reduced pressure gave a dark green thickish residue, which was partitioned between ethyl acetate and water. The ethyl acetate layer was repeatedly extracted out with 4% Na_2CO_3 to separate the acidic and the neutral fractions. The Na_2CO_3 layer was acidified with dilute HCl and shaken out with ethyl acetate which was washed, dried and charcoaled. The filtrate along with the ethyl acetate eluate of the charcoal was freed of the solvent and the viscous residue was divided into petroleum ether soluble and insoluble fractions. On preparative tlc (silica gel; benzene-acetone 8:2), the latter fraction yielded three components **A-1**, **A-2** and **A-3**, the last two being the major constituents. **A-1**, after purification through preparative tlc (silica gel; chloroform-methanol 95:5) and crystallization from chloroform afforded

isoazadirolide (**I**) as rods, mp 130°C, $[\alpha]^{24}_{D}$ = 200° ($CHCl_3$). HRMS m/z (%): 586.2738 (M^+, calcd. for $C_{32}H_{42}O_{10}$: 586.2776)(3), 558.2807 (M-CO) (4), 524.2765 (M-CO_2-H_2O) (6), 486.2266 (M- >C=CHCOOH) (6) and 424.2251 ($C_{26}H_{32}O_5$) (10).

A-2 which crystallized from chloroform as fine needles, mp 204°C, was identified as scopoletin through comparison of its physical and spectral data with those reported in literature[10,11]. **A-3** was ultimately resolved into four constituents isonimocinolide[5], isonimbocinolide[8], nimocinolide[5] and nimbocinolide[9], communicated earlier.

Acetylation of I to II

To a solution of **I** (4 mg) in pyridine (1 ml), acetic anhydride (2 ml) was added and the reaction mixture kept overnight at room temperature. On usual work up, **II** was obtained as a crystalline residue which on recrystallization from ethyl acetate formed irregular plates, mp 105°C; uv λ_{max} (MeOH) nm: 210, 294; ir ν_{max} ($CHCl_3$) cm^{-1}: 1760 (α,β-unsaturated-γ-lactone), 1742 (carbomethoxyl), 1725 (br, ester carbonyls), 1640 and 820 (trisubstituted double bond), 1140 and 1080 (ether linkage). HRMS m/z (%): 670.2942 (M^+, calcd. for $C_{36}H_{46}O_{12}$: 670.2987) (2), 610.2756 (M-$C_2H_4O_2$) (5), 550.2550 (M-2x$C_2H_4O_2$) (4), 528.2707 (M-side chain) (10) and 468.2577 (M-side chain - $C_2H_4O_2$) (20). ^{1}H-nmr (300 MHz, $CDCl_3$) δ : 6.89 (1H, m, H-21), 6.09 (1H, m, H-22), 6.07 (1H, s, H-2'), 5.63 (1H, m, $W_{\frac{1}{2}}$ = 7.0 Hz, H-1), 5.44 (1H, m, $W_{\frac{1}{2}}$ = 6.9 Hz, H-3), 5.36 (1H, m, H-15), 4.18 (1H, d, J = 3.3 Hz, H-7), 3.96 (1H, dd, J = 12.6 and 3.3 Hz, H-6), 3.70 (1H, d, J = 7.2 Hz, H-28α), 3.56 (1H, d, J = 7.2 Hz, H-28β), 3.47 (3H, s, OMe), 3.44 (1H, d, J = 9.0 Hz, H-17), 2.72 (1H, d, J = 12.6 Hz, H-5), 2.65 (1H, t, $J_{9,11\alpha}$ = $J_{9,11\beta}$ = 6.0 Hz, H-9), 2.43 (1H, dd, J_{gem} = 12.6 Hz, $J_{16\alpha,15}$ = 6.4 Hz, H-16α), 2.36 (1H, dd, J_{gem} = 16.0 Hz, $J_{11\beta,9}$ = 6.0 Hz, H-11β), 2.27 (1H, m, H-2β), 2.20 (1H, m, H-11α), 2.15 (1H, m, H-16β), 2.03 (1H, m, H-2α), 2.17 and 1.92 (each 3H, br.s, >C=CHCOO), 1.98 and 1.97 (each 3H, s, 2xOAc), 1.71 (3H, d, J = 1.2 Hz, H-18), 1.28 (3H, s, H-30), 1.25 (3H, s, H-29) and 1.19 (3H, s, H-19).

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