ISONIMOLIDE AND ISOLIMBOLIDE, TWO NEW TETRANORTRITERPENOIDS FROM THE TWIGS OF *AZADIRACHTA INDICA* A.JUSS (MELIACEAE)

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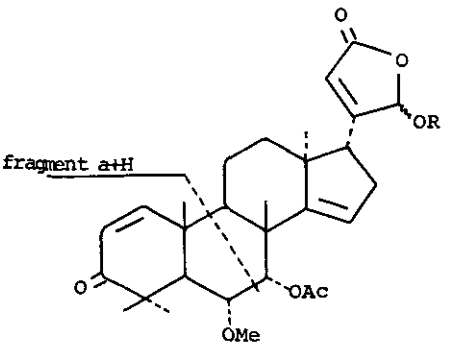
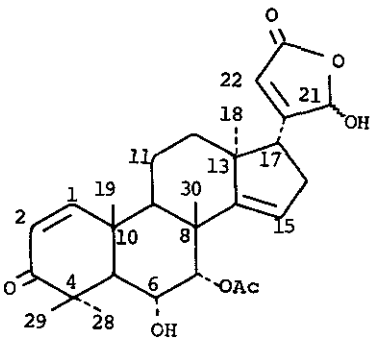
Abstract - Two new tetranortriterpenoid γ-hydroxybutenolides named as isonimolide and isolimbolide along with isonimocinolide, have been isolated from the acidic fraction of the fresh, undried, spring twigs of *Azadirachta indica* (neem). The structures of these limonoids have been established through chemical and spectral studies.

Several new triterpenoids have been reported earlier by Siddiqui et al., as a result of isolation and structure elucidation studies in the terpenoidal constituents of the fresh fruits[1-6] leaves[6-10] and the twigs[11,12] of *Azadirachta indica* (neem). In continuation of these studies, two new tetranortriterpenoid γ-hydroxybutenolides named as isonimolide (I) and isolimbolide (IV) have been isolated from the acidic fraction of the fresh, undried, spring twigs, together with isonimocinolide (III), isolated earlier from the leaves.[6] The structures of these constituents have been elucidated through chemical and spectral studies.

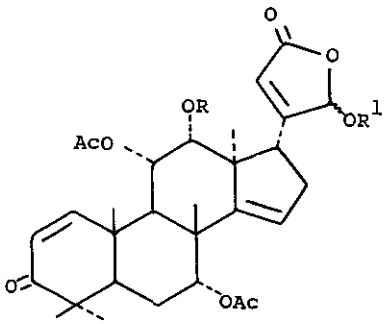
Isonimolide (I) has molecular formula $C_{29}H_{38}O_7$ (high resolution mass). Its uv spectrum showed maxima at 208 (ε 3141) and 285 nm (ε 306), while the ir spectrum exhibited peaks at 3400 (OH), 1760 (α,β-unsaturated γ-lactone), 1725 (ester carbonyl), 1665 (cyclohexenone), 1650 and 820 (trisubstituted double bond) and 1100 cm^{-1} (OMe). The mass and ^{1}H-nmr (vide experimental) spectra of I showed that it has a tetracyclic carbocyclic nucleus with a 21-hydroxybut-20(22)-ene-γ-lactone side chain. The ^{1}H-nmr spectrum further indicated the presence of 1-en-3-one system in ring-A, a C=C at C-14 and substituents at both C-6 and C-7, one representing the acetoxy function (δ 2.04). The chemical shift and coupling constant of H-7 (δ 5.34, J=2.4Hz) showed that C-7 carries an acetoxy function with α orientation. A comparison of the molecular formula and the spectral data of I, with those of isonimocinolide (III),[6] showed that I is very similar to III and only has an increment of a CH_2 moiety. This was taken for a methoxy group, instead of the hydroxyl function at C-6 in III, in the light of the ^{1}H-nmr



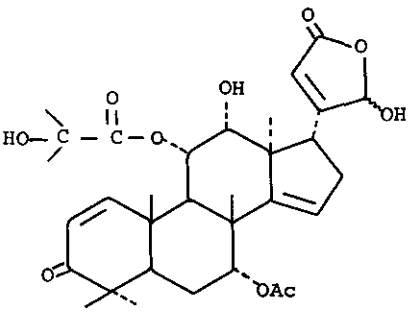
I: R=H
II: R=OAc



Isonimocinolide (III)



IV: R=R^1=H
V: R=R^1=Ac



Isonimbocinolide (VI)

spectrum which exhibited a three-proton singlet at δ 3.63, and formation of only the monoacetyl derivative II, on reaction with acetic anhydride-pyridine. This was corroborated by the coupling constants of H-6 (δ 4.36, dd, J=11.5 and 2.4Hz) and the mass spectrum of I, which showed a diagnostic fragment at m/z 181.1220 (fragment a+H), resulting from the cleavage of ring-B. In the ^{1}H-nmr spectrum of II (vide experimental), the signal corresponding to H-21 shifted to δ 6.87 along with the appearance of only two acetoxy methyl functions at δ 2.17 and 2.04. In the light of these observations, isonimolide has been assigned structure I.

Isolimbolide (IV) has molecular formula $C_{30}H_{38}O_9$ (high resolution mass) and showed uv absorption at 210 nm (ε 9756). The ir spectrum showed peaks at 3450 (OH), 1760 (α,β-unsaturated γ-lactone), 1740 and 1720 (ester carbonyls), 1660 (cyclohexenone), 1640, and 820 cm^{-1} (trisubstituted double bond). The triterpenoidal nature of IV was indicated by the appearance of five methyl signals in the ^{1}H-nmr spectrum (vide

experimental). Other significant features, exhibited by the spectral data are: 1-en-3-one system in ring-A, a C=C at C-14, two acetoxy and one hydroxy functions, and a 21-hydroxybut-20(22)-ene-γ-lactone side chain. The chemical shift and the width of the multiplet at half height of H-7 (δ 5.34, $W_{\frac{1}{2}}$ = 6.5Hz) showed that one of the acetoxy functions is located at C-7.[13] The ^{1}H-nmr spectrum further demonstrated a one-proton double doublet at δ 5.46 (J=12.5 and 2.9Hz) and a one-proton doublet at δ 4.06 (J=2.9Hz). These signals implied that the second acetoxy function and the hydroxyl group are located at C-11 and C-12 respectively both with α disposition. Acetylation of IV with acetic anhydride-pyridine afforded the acetyl derivative V, in the ^{1}H-nmr spectrum of which (vide experimental), the signals of H-21 and H-12 shifted to δ 6.87 and 5.42 respectively, along with the appearance of four acetoxy methyl signals at δ 2.17, 2.03 (6H) and 2.00. In the light of these observations, structure IV has been assigned to isolimbolide, which was corroborated by the high resolution mass spectrum which showed significant fragments at m/z 482.2365 (M-$C_2H_4O_2$), 442.2343 (M-side chain), 422.2100 (M-2x$C_2H_4O_2$), and 137.0929 ($C_9H_{13}O$, ring-A+H). It may be mentioned that IV has a close structural relationship with isonimbocinolide (VI),[9] with a variation in the composition of the side chain at C-11, which is 2-methyl-2-hydroxypropionate in VI.

The stereochemistry of various centres of isolimbolide (IV) has been established through NOESY which showed the spatial connectivities of H-1 with H-2; H-11 with H-12; and H-17 with H-12 and H-16β. The latter interaction exhibited that the side chain at C-17 is α oriented.

The butenolides I and IV as well as isonimocinolide (III) were also detected in the fresh methylene chloride extract of the twigs, which showed that they are genuine natural products and not artifacts. Further, as other γ-hydroxy-butenolides have been shown to possess insect growth regulating[6] and insect antifeeding[14] properties, I and IV may on biological evaluation, prove to share these properties. Isonimolide (I) is closely related to isonimocinolide (III)[6] and may be transformed into the latter through demethylation. On the other hand, since the hydroxybutenolide side chain has been considered as the intermediate in the formation of furan ring in meliacins,[15] I and III may be regarded as the precursors in the biosynthesis of nimocinol.[7] Further, isolimbolide (IV)

may be considered as an intermediate in the biosynthesis of 11α-acetoxyazadirone,[16] since the latter can be obtained from IV through the transformation of the hydroxybutenolide side chain to the furan ring, and dehydroxylation of the hydroxyl group at C-12.

EXPERIMENTAL

Melting points were recorded in the 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. ^{1}H-nmr spectra and NOESY experiments (pulse delay 2 sec., mixing time 0.5 sec.) were run on Bruker AM 300 NMR spectrometer. Merck Kieselgel 60 PF_{254} and Aluminium oxide PF_{254} coated on glass plates were used for analytical (thin layer) and preparative (thick layer) chromatography.

Isolation of Tetranortriterpenoids

The fresh, undried, uncrushed neem twigs, collected in spring from Karachi region, were repeatedly percolated with methylene chloride at room temperature. The methylene chloride and the aqueous layers were separated from the combined percolates and the former being freed of the solvent under reduced pressure. The dark brownish residue was shaken up with ethyl acetate and water and the ethyl acetate layer was repeatedly extracted out with 4% Na_2CO_3 to separate the acidic and neutral fractions. The combined Na_2CO_3 phase was acidified with dilute HCl and extracted out with ethyl acetate, which was washed, dried (Na_2SO_4 anhydrous) and charcoaled. The filtrate along with the ethyl acetate eluate of the charcoal was freed of the solvent and the residue was subjected to preparative tlc (silica gel; chloroform-methanol 98:2) which afforded a major band A along with some minor constituents. Band A was resolved into three bands A-1, A-2 and A-3 through preparative tlc (silica gel; benzene-ethyl acetate 5:95) and band A-1 was ultimately divided into isonimolide (I), isonimocinolide (III) and isolimbolide (IV), through preparative tlc on plates coated with aluminium oxide (chloroform-methanol 97:3). A-2 afforded desacetylnimbinolide, desacetylisonimbinolide and desacetylnimbin[12] while A-3 was resolved into margosinolide and isomargosinolide,[11] communicated earlier.

Isonimolide (I)

On recrystallization from methanol, I formed bunches of irregular plates, mp 145-148°C, $[\alpha]_D^{22}$ = 50°(c=0.04,$CHCl_3$). HRMS m/z (%): 498.2631 (M^+, calcd. for $C_{29}H_{38}O_7$: 498.2616) (5), 483.2319 (M-CH_3) (4), 466.2361 (M-CH_3OH) (10), 438.2420 (M-$C_2H_4O_2$) (8), 398.2447 (M-side chain) (6), 181.1220 (fragment a+H) (15) and 137.0970 (ring-A+H) (32). ^{1}H-nmr (300 MHz, $CDCl_3$) δ : 7.01 (1H, d, $J_{1,2}$=10.1Hz, H-1), 5.99 (1H, m, H-22), 5.93 (1H, m, H-21), 5.90 (1H, d, $J_{2,1}$=10.1Hz, H-2), 5.42 (1H, m, H-15), 5.34 (1H, d, $J_{7,6}$ =2.4Hz, H-7), 4.36 (1H, dd, $J_{6,5}$=11.5Hz, $J_{6,7}$=2.4Hz, H-6), 3.63 (3H, s, OMe), 2.87 (1H, m, H-11α), 2.75 (1H, m, H-12β), 2.49 (1H, m, H-12α), 2.34 (1H, m, OH), 2.20 (1H, d, $J_{5,6}$=11.5Hz, H-5), 2.15 (1H, dd, $J_{9,11\beta}$=11.0Hz, $J_{9,11\alpha}$=2.9Hz, H-9), 2.12 (1H, m, H-17), 2.07 (1H, m, H-11β), 2.04 (3H, s, OAc), 2.00-1.90 (2H, m, H-16), 1.40, 1.31, 1.28, 1.27 and 1.12 (each 3H, s, δxCH_3).

Isolimbolide (IV)

It crystallized from chloroform as colourless plates, mp 92-94°C, $[\alpha]_D^{22}$ = 33.3°(c=0.03, $CHCl_3$). HRMS m/z (%): 542.2520 (M^+, calcd. for $C_{30}H_{38}O_9$: 542.2514) (2), 482.2365 (M-$C_2H_4O_2$) (5), 442.2343 (M-side chain) (8), 422.2100 (M-2x$C_2H_4O_2$) (6) and 137.0929 (ring-A+H) (60). ^{1}H-nmr (300 MHz, $CDCl_3$) δ : 7.08 (1H, d, $J_{1,2}$=10.0Hz, H-1), 6.00 (1H, m, H-22), 5.95 (1H, m, H-21), 5.90 (1H, d, $J_{2,1}$=10.0Hz, H-2), 5.46 (1H, dd, $J_{11\beta,9}$=12.5Hz, $J_{11\beta,12\beta}$=2.9Hz, H-11β), 5.42 (1H, m, H-15), 5.34 (1H, m, $W_{\frac{1}{2}}$ = 6.5Hz, H-7), 4.06 (1H, d, $J_{12\beta,11\beta}$=2.9Hz, H-12β), 3.55 (2H, m, 2xOH), 2.91 (1H, ddd, $J_{17,16\beta}$=9.3Hz, $J_{17,16\alpha}$=7.5Hz, $J_{17,22}$=1.8Hz, H-17), 2.73 (1H, d, $J_{9,11\beta}$=12.5Hz, H-9), 2.60 (1H, ddd, J_{gem}=16.0Hz, $J_{16\alpha,17}$=7.5Hz, $J_{16\alpha,15}$=1.8Hz, H-16α), 2.46 (1H, m, H-16β), 2.17 and 2.04 (each 3H, s, 2xOAc), 1.91 (1H, m, H-5), 1.79 (2H, m, H-6), 1.31, 1.28, 1.26, 1.19 and 1.16 (each 3H, s, 5xCH_3).

Acetylation of I to II

To a solution of I (3 mg) in pyridine (0.5ml), acetic anhydride (1 ml) was added and the reaction mixture kept overnight at room temperature. On usual work up, II was obtained as crystalline product which on recrystallization from chloroform formed prismatic rods, mp 118°C; uv λ_{max}(MeOH) nm: 212 (ε 5260); ir ν_{max}($CHCl_3$) cm^{-1}: 1765, 1725, 1665, 1650, 1100 and 825. HRMS m/z (%): 540.2756 (M^+, calcd. for $C_{31}H_{40}O_8$: 540.2722) (2), 480.2571 (M-$C_2H_4O_2$) (5), 420.2350 (M-2x$C_2H_4O_2$) (15) and 398.2400 (M-side chain) (8). ^{1}H-nmr (300 MHz, $CDCl_3$) δ : 7.09 (1H, d, $J_{1,2}$= 10.2Hz, H-1), 6.87 (1H, m, H-21), 6.00 (1H, m, H-22), 5.93 (1H, d, $J_{2,1}$=10.2Hz, H-2), 5.44 (1H, m, H-15), 5.36 (1H, d, $J_{7,6}$=2.5Hz, H-7), 4.36 (1H, dd, $J_{6,5}$=11.8Hz,

$J_{6,7}$=2.5Hz, H-6), 3.64 (3H, s, OMe), 2.80-2.50 (3H, m, H-11α,H-12α and H-12β), 2.48 (1H, d, $J_{5,6}$=11.8Hz, H-5), 2.31 (1H, dd, $J_{9,11\beta}$=11.0Hz, $J_{9,11\alpha}$=3.0Hz, H-9), 2.25 (1H, m, H-16β), 2.17 (3H, s, OAc), 2.14 (1H, m, H-17), 2.10 (1H, m, H-16α), 2.04 (3H, s, OAc), 1.72 (1H, m, H-11β), 1.32, 1.24, 1.18, 1.15 and 1.09 (each 3H, s, 5xCH_3).

Acetylation of IV to V

Acetylation of IV (2mg) was carried out in the same manner as in case of I. After usual work up and recrystallization from chloroform, V was obtained as bunches of needles, mp 76-78°C; uv λ_{max}(MeOH) nm: 212 (ε5215); ir ν_{max}($CHCl_3$) cm^{-1}: 1760, 1740-1720, 1665, 1640 and 825. HRMS m/z (%): 626.2785 (M^+, calcd. for $C_{34}H_{42}O_{11}$: 626.2725) (2), 506.2330 (M-2x$C_2H_4O_2$) (10), 484.2485 (M-side chain) (5) and 386.1800 (M-4x$C_2H_4O_2$) (12). ^{1}H-nmr (300 MHz, $CDCl_3$) δ : 7.10 (1H, d, $J_{1,2}$=10.0Hz, H-1), 6.87 (1H, m, H-21), 6.00 (1H, m, H-22), 5.93 (1H, d, $J_{2,1}$=10.0Hz, H-2), 5.44 (1H, m, H-15), 5.42 (1H, d, $J_{12\beta,11\beta}$=2.5Hz, H-12β), 5.38 (1H, dd, $J_{11\beta,9}$=12.0Hz, $J_{11\beta,12\beta}$=2.5Hz, H-11β), 5.34 (1H, m, $W_{½}$=7.5Hz, H-7), 2.90-2.60 (2H, m, H-16α and H-17), 2.50 (1H, d, $J_{9,11\beta}$=12.0Hz, H-9), 2.39 (1H, m, H-16β), 1.94 (1H, m, H-5), 1.70 (2H, m, H-6), 2.17(3H), 2.03(6H), 2.00(3H) (each s, 4xOAc), 1.32(3H), 1.25(6H), 1.18(3H) and 1.17(3H) (each s, 5xCH_3).

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