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Constituents of *Limonia acidissima*. Applications of Two-dimensional NMR Spectroscopy in Structure Elucidation

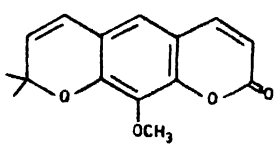
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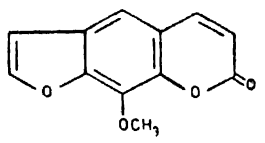
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The leaves and stems of *Limonia acidissima* afforded coumarins (luvangetin, xanthotoxin and marmesin), triterpenoids (lupeol and limonin) and steroids (sitosterol and sitosterol-*O*-β-D-glucoside). These compounds have been characterised by their spectral (uv, ir, ^{1}H- and ^{13}C-nmr, mass) characteristics. Detailed proton and carbon-13 spectral analyses of limonin have been made using two-dimensional nmr spectroscopy.

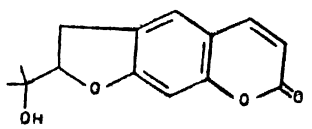
LIMONIA acidissima L.[1] is reported to have anti-epileptic, purgative and sudorific properties, and used for the cure of colic trouble and cardialgia[2]. As a part of our phytochemical investigation we undertook chemical investigation of the plant material collected locally. Luvangetin (**1**), xanthotoxin (**2**), marmesin (**3**), lupeol, limonin (**4**), sitosterol and its *O*-β-D-glucoside were isolated and characterised by their uv, ir, ^{1}H- and ^{13}C-nmr and mass spectral characteristics and finally by direct comparison with authentic samples.



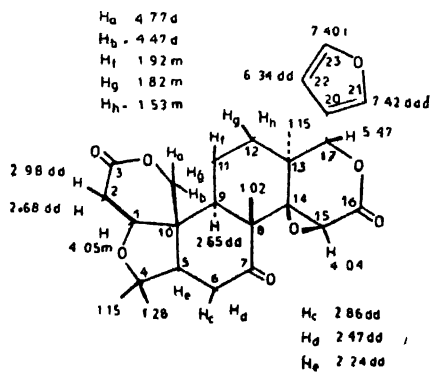
(1)



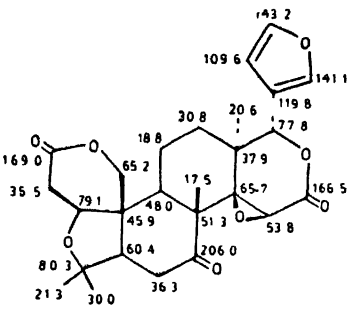
(2)



(3)



(4)



(4) ^{13}C-nmr

Detailed proton resonance assignment of limonin given around expression **4** was made from extensive homonuclear decoupling as well as two-dimensional homonuclear proton shift correlation as shown in Fig. 1. Homodecoupling experiments established that the signal at δ 4.05 (1H, m) is vicinally coupled with the resonances at δ 2.68 (1H, dd, J_{vic} 1.8 Hz) and 2 98 (1H, dd, J_{vic} 4.0 Hz) which are further coupled to each other geminally (J_{gem} 17.0 Hz). Similarly, the signals at δ 4.47 (1H, d, J 13.1 Hz) and 4.77 (1H, d, J 13 1 Hz) could also be related to a set of nonequivalent geminal protons on carbon bearing lactone function. The resonance at δ 2.55 (1H, dd, J 12.1 and 2.8 Hz) was coupling with complex signals in the region δ 1.80 – 1.95. Though decoupling experiments established the presence of three mutually interacting nuclei resonating at δ 2 24 (1H, dd, J 15.8 and 3.3 Hz), 2.47 (1H, dd, J 14.6 and 3.3 Hz) and 2.86 (1H, dd, J 15.8 and 14 6 Hz) it was not possible to make their specific assignments. HETCOR experiment (*vide infra*) established the association of the resonance at δ 2.24 (dd) with C-5 while C-6 was linked with protons resonating at δ 2.47 (dd) and 2.86 (dd). Coupling pattern of the signals at δ 6.34 (1H, dd, J 1.7 and 0.8 Hz), 7.40 (1H, t, J 1.7 Hz) and 7.42 (1H, ddd, J 1.7, 0.8 and 0.8 Hz) further established their association with H-22, H-23 and H-21, respectively.

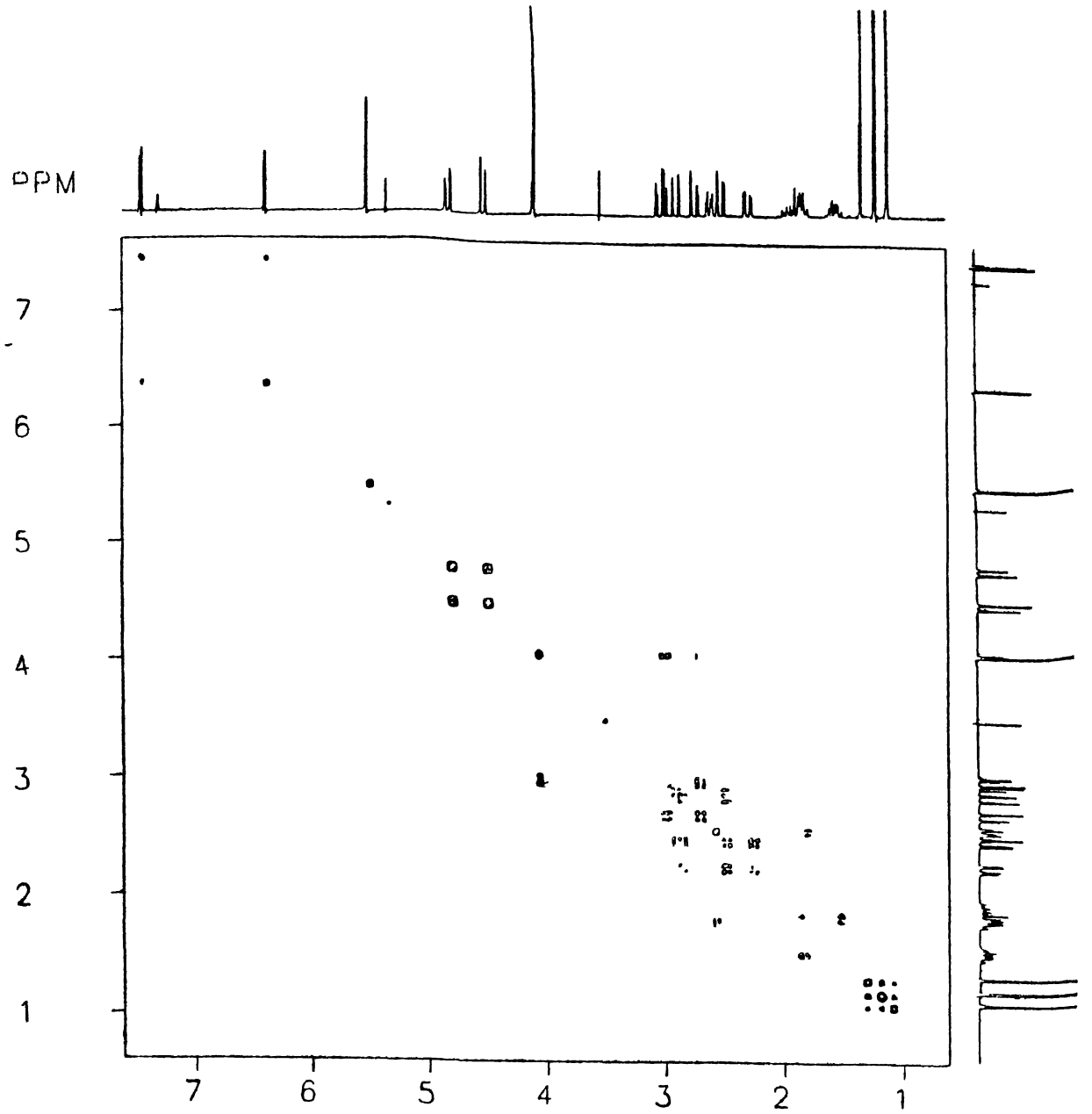


Fig. 1

The homonuclear contour plot was obtained by pulsing the sample with radiofrequency and accumulating the data after certain incremental time intervals and application of magnetisation transfer pulse[3,4]. This follows a discret FID pattern which is Fourier transformed to get the plot as shown in Fig. 1. The spectra is along diagonal and the off-diagonal peaks are for coupled signals[3,4]. HOMCOR plot not only supported the findings from homodecoupling experiments described above but also gave useful additional information. Thus, both δ 1.53 and 2.55 resonances are found to couple with resonances at δ 1.82 (m) and 1.92 (m). The contour plot also indicated interaction between the proton signals at δ 5.47 (1H, s) and 7.42 (1H, ddd) as well as between δ 5.47 and 1.15 (6H, s). Interactions between methyls at δ 1.15 (6H, s) and 1.02, 1.02 and 1.28, and also between methyls resonating at δ 1.15 and 1.28 established assignments as shown.

The 2D-contour HETCOR ($^{13}C-^{1}H$) plot (Fig. 2) helped in completing the resonance assignments for limonin[5] (4). This experiment is a slightly modified version of INEPT experiment and uses an additional delay ($1/2J$) before the applica-

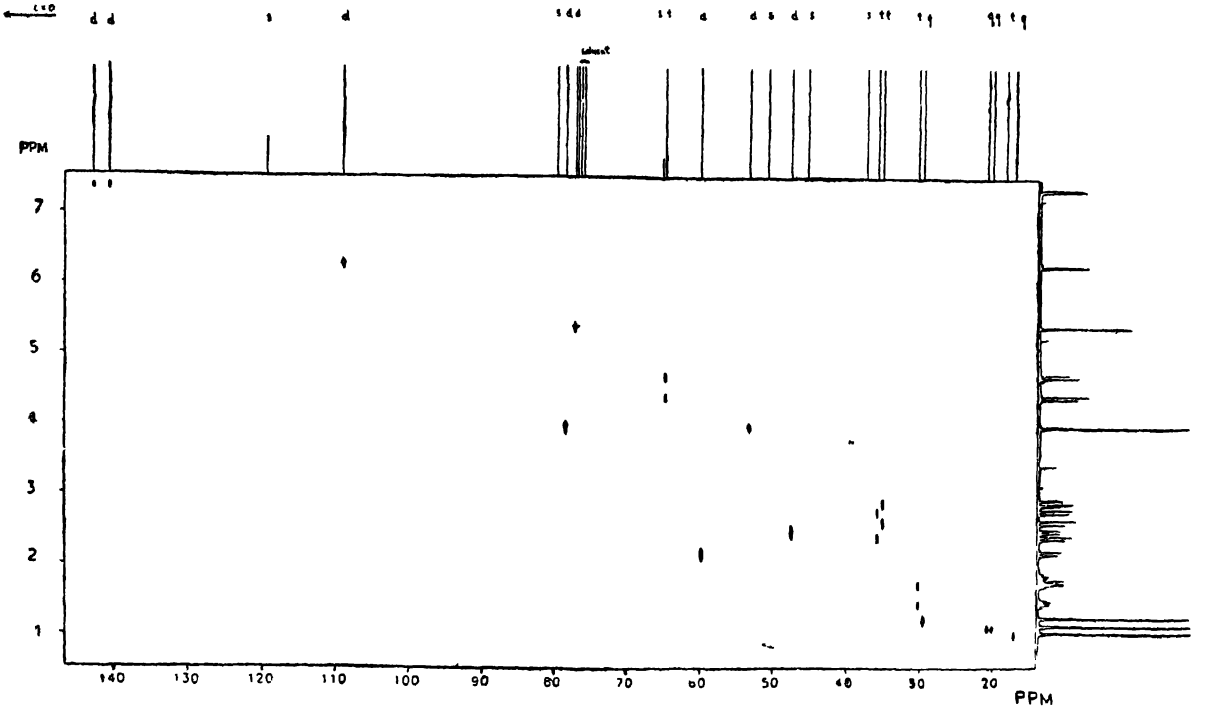


Fig. 2

tion of the proton polarisation transfer pulse[3,4]. The net result is the appearance of a spot (in contour plot) correlating the carbon signal and the signal for proton(s) directly linked with. Thus, correlation of methine carbon signal at δ 79.1 with proton signal at δ 4.05 (1H, m) not only identified the relation of the former with C-1 but also differentiated it from the other methine carbon resonance at δ 77.8, correlating with proton resonance at δ 5.47 (1H, s), associated to C-17. Similarly, the methine carbon signal at δ 53.8 correlating with singlet proton resonance at δ 4.04 is assignable to C-15 whereas the methine carbon resonance at δ 60.4 correlating with the proton resonance at δ 2.24 (dd) with C-5. Thus, C-9 resonates at δ 48.0. Identification of the carbon signals associated with furan ring are made similarly. The proton resonances at δ 6.34 (H-22), 7.40 (H-23) and 7.42 (H-21) are correlated with carbon resonances at δ 109.6 (C-22), 143.2 (C-23) and 141.1 (C-21), respectively.

The δ 65.2 resonance could be related straightaway with methylene carbon bearing lactone moiety. The methylene carbon resonances at δ 35.5 and 36.3 are likewise distinguished and related to C-2 and C-6, respectively. Again, δ 18.8 resonance related with proton resonances at δ 1.82 and 1.92 is for C-11 while δ 30.8 signal correlating with proton resonances at δ 1.53 and 1.82 is for C-12.

Methyl carbon signals at δ 30.0, 21.3, 20.6 and 17.5 are linked with the protons resonating at δ 1.28, 1.15, 1.15 and 1.02 (3H each, singlets) and are thus associated with C-24, C-25, C-19 and C-18, respectively. Distinction between non-protonated carbon resonances for C-8, C-10 and C-13 could be made by considering α- and β-effects, and effect of carbonyl function. C-10 has one more β-effect than C-13 while C-8 has one less β-effect than C-10 but experiences strong deshielding effect of carbonyl group. Heteronuclear 2D contour plot (J 7 Hz) for two and three bond couplings gave further support to the assignments for non-protonated carbons made as above. Thus, δ 206.0, 51.3 and 37.9 resonances correlated with methyl resonances at δ 1.02, 1.02 and 1.15, respectively, while carbon signal at δ 80.3 correlated with methyl resonances at δ 1.28 and 1.15, and δ 65.7 singlet carbon with methyl resonances both at δ 1.02 and 1.15. Again, C-16 carbon resonance at δ 166.5 was related with a proton resonance at δ 4.04.

Experimental

M.ps. are uncorrected. Ir spectra were taken in KBr, rotations in $CHCl_3$, nmr spectra in $CDCl_3$. Proton noise decoupling and attached proton test experiments established carbon shifts and degree of protonation. The solvent served as internal lock and internal standard ($\delta_{TMS} = \delta_{CDCl_3} + 76.9$ ppm).

The ethanol extract of the whole plant (5 kg) was chromatographed over silica gel and eluted with solvents and the solvent mixtures of increasing polarity. Petrol–benzene (1 : 1) eluates afforded lupeol. Benzene eluates afforded luvangetin (**1**), xanthotoxin (**2**) and sitosterol in succession.

Chloroform eluates afforded marmesin (3) and limonin (4). Chloroform–methanol (85 : 15) eluates yielded sitosterol-*O*-β-D-glucoside. The compounds were purified by repeated chromatography.

Luvangetin (1) : $C_{15}H_{14}O_4$, m.p. 102° ($CHCl_3$–petrol) ; ν_{max} 1 730 cm^{-1} ; ^1H nmr δ 1.51 (6H, s, >CMe_2), 3.97 (3H, s, OCH_3), 5.69 (1H, d, *J* 9.9 Hz, H-2′), 6.22 (1H, d, *J* 9.5 Hz, H-3), 6.33 (1H, d, *J* 9 9 Hz, H-1′), 6.82 (1H, s, H-5), 7.55 (1H, d, *J* 9.5 Hz, H-4) ; ^{13}C nmr δ 27.8 (C-4′,5′), 61.0 (OCH_3), 77.4 (C-3′), 112.6 (C-4a), 112.7 (C-3), 118.8 (C-6), 118.9 (C-1′), 120.7 (C-5), 130.8 (C-2′), 135 2 (C-8), 143.4 (C-4), 147.8 (C-8a), 148.9 (C-7), 160.2 (C-2) ; *m/z* (%) 258 (M^+, 17), 243 (61), 217 (15), 216 (100), 201 (23), 173 (34) and 145 (13).

Xanthotoxin (2) : $C_{12}H_8O_4$, m.p. 144° ($CHCl_3$–petrol) ; ν_{max} 1 710 cm^{-1} ; ^1H nmr δ 4.30 (3H, s, OCH_3), 6.35 (1H, d, *J* 9.5 Hz, H-3), 6.81 (1H, d, *J* 2.2 Hz, H-3′), 7.35 (1H, s, H-5), 7.68 (1H, d, *J* 2.2 Hz, H-2′), 7.75 (1H, d, *J* 9.5 Hz, H-4) ; ^{13}C nmr δ 61.1 (OMe), 106.6 (C-3′), 112.8 (C-3), 114.5 (C-5), 116.3 (C-4a), 126.0 (C-6,8), 139.2 (C-8a), 144.2 (C-4), 145.0 (C-7), 146.5 (C-2′) and 160.5 (C-2).

Marmesin (3) : $C_{14}H_{14}O_4$, m.p. 192° ($CHCl_3$–petrol) ; $[\alpha]_D^{25}$ +26° ; ν_{max} 3 450 and 1 702 cm^{-1} ; ^1H nmr δ 1.23 and 1.37 (>CMe_2), 1.85 (1H, br s, exchangeable with D_2O, OH), 3.23 (2H, br d, *J* 8.8 Hz, H_2-1′), 4.74 (1H, t, *J* 8.8 Hz, H-2′), 6.21 (1H, d, *J* 9.5 Hz, H-3), 6.74 (1H, s, H-8), 7.22 (1H, s, H-5), 7.59 (1H, d, *J* 9.5 Hz, H-4) ; *m/z* (%) 246 (M^+, 39), 213 (20), 188 (75), 187 (100), 175 (15), 160 (30), 131 (19), 59 (66) and 43 (17).

Lupeol : $C_{30}H_{50}O$ (M^+ 426) ; m.p. 212° ($CHCl_3$–petrol) ; $[\alpha]_D^{25}$ +27° ; ν_{max} 3 335 cm^{-1} ; ^{13}C nmr δ 14.4 (C-27), 15.3 (C-24), 15.9 (C-26), 16.0 (C-25), 17.9 (C-28), 18.2 (C-6), 19.2 (C-30), 20.8 (C-11), 25 0 (C-12), 27.3 (C-2,15), 27.9 (C-23), 29.8 (C-21), 34.2 (C-7), 35.5 (C-16), 37.0 (C-10), 38.0 (C-13), 38.6 (C-1), 38 7 (C-4), 39 9 (C-22), 40.7 (C-8), 42.7 (C-14), 42.9 (C-17), 47 9 (C-19), 48.2 (C-18), 50 3 (C-9), 55.2 (C-5), 78.8 (C-3), 109.2 (C-29) and 150.8 C-20).

Limonin (4) : $C_{26}H_{30}O_8$, m.p. 298° ($CHCl_3$) ; $[\alpha]_D^{25}$ –125° ; ν_{max} 1 760, 1 712 and 878 cm^{-1} ; *m/z* (%) 470 (M^+, 1), 413 (4), 348 (21), 347 (100), 331 (5), 135 (25), 108 (23) and 95 (32).

Sitosterol : $C_{29}H_{50}O$, m.p. 136–37° ($CHCl_3$–petrol) ; $[\alpha]_D^{25}$ +34° ; ν_{max} 3 400 cm^{-1}.

Sitosterol-O-β-D-glucoside : ν_{max} 3 350 cm^{-1} (br) ; with Ac_2O/pyridine formed tetraacetate. $C_{42}H_{66}O_8$ (M^+ 744 5) ; m.p. 148–49° ($CHCl_3$–MeOH) ; $[\alpha]_D^{25}$ –29° ; ν_{max} 1 752, 1 740, 1 252 and 1 215 cm^{-1}.

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