Phytochemistry, Vol. 28, No 6, pp 1703–1710, 1989
Printed in Great Britain

0031-9422/89 $3 00+0 00

CONFIGURATIONAL STUDIES ON HYDROXY GROUPS AT C-2, 3 AND 23 OR 24 OF OLEANENE AND URSENE-TYPE TRITERPENES BY NMR SPECTROSCOPY*

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(*Received in revised form* 12 *October* 1988)

Key Word Index—Ursene triterpenoids; oleanene triterpenoids; ^{1}H NMR, ^{13}C NMR, configurational analysis, chemotaxonomy.

Abstract—Configurational determination of 2,3-dihydroxy and 2,3,23- and 2,3,24-trihydroxy substituents in triterpenoids by ^{1}H NMR spectroscopy can be unambiguously carried out by analysing signal peaks of the protons on oxygen-bearing carbon atoms. The application of our results leads to the revision of a few triterpene structures previously reported.

INTRODUCTION

In the early 1960's attempts were made to assign resonance positions to the methyl groups in the ^{1}H NMR spectra of various triterpenoids [2–8]. By 1967 the assignment of all methyl groups of the oleanene type was settled and the effects of various substituents on their chemical shifts were demonstrated, to a first approximation, to be additive [9, 10]. This concept was extended to the ursene ring systems and other functions [11–14]. The methyl peaks are readily discernible as sharp singlet or doublet absorptions, and this fact has been made use of in the structure determination of unknown oleanene and ursene triterpenes [15–19]. However, in some cases the assignments were obscure or, at worst, led to incorrect structures.

Further evidence for structure assignment came from analysis of signals due to protons alpha to hydroxy or acetoxy groups at C-2 and C-3 [12, 13]. The assignment of the acetoxy-bearing methylene protons at C-23 or C-24 has been reported [20]. This paper supplies the ^{1}H and ^{13}C NMR spectral data of methyl 2β,3α-dihydroxyurs-12-en-28-oate and its diacetate, and compares them to the data obtained for three isomers. In addition, it evaluates the additional effects of oxygen at C-23 or C-24 on the protons at C-2 and C-3. These results have been used to revise some triterpene structures already reported as novel compounds.

RESULTS AND DISCUSSION

Methyl 2,3-*dihydroxyurs*-12-*en*-28-*oates and their diacetates*‡

The ^{1}H and ^{13}C NMR spectral data of four dihydroxy and four diacetoxy configurational isomers are shown in Tables 1 and 2. The spectral patterns of 2β,3α-$(OH)_2$ and -$(OAc)_2$ are clearly different from those of the other three pairs, because of the boat or twist conformation of their A rings [13, 21].

Though the order of the positions of the chemical shifts of angular methyl groups is the same as calculated by Biessels *et al.* [15], the differences between our observed chemical shifts and their calculated ones are more than 0.02 ppm for the 23- and 25-methyl groups in 2α,3α-$(OH)_2$, the 23-, 25- and 26-methyl groups in 2α,3β-$(OH)_2$, the 23- and 26-methyl groups in 2β,3β-$(OH)_2$ and at all angular methyl signals in 2β,3α-$(OH)_2$. The effects of acetylation on the 23-, 24- and 25-methyl signals indicate that the 23- methyl group is shielded (*ca* 0.1 ppm) and the 24-methyl is deshielded (*ca* 0 1 ppm) except for 2β,3α-$(OAc)_2$, whereas the 25-methyl is deshielded (*ca* 0.05 ppm) except for 2β,3β-$(OAc)_2$.

The distances ($\Delta\delta_{2-3}$) between the chemical shifts of H-2 and H-3 are 0.88 ppm in 2β,3β-$(OH)_2$ and 0 7 ppm in 2β,3β-$(OAc)_2$ > 0.69 ppm in 2α,3β-$(OH)_2$ and 0.35 ppm in 2α,3β-$(OAc)_2$ > 0.57 ppm in 2α,3α-$(OH)_2$ and 0.27 ppm in 2α,3α-$(OAc)_2$ > 0.12 ppm in 2β,3α-$(OH)_2$ and −0.08 ppm in 2β,3α-$(OAc)_2$. The figures for the dihydroxy compounds are larger (*ca* 0.2 to 0.3 ppm) than those of the corresponding diacetates because of the lower downfield shift of the H-3 on acetylation. Though the $\Delta\delta_{2-3}$ values in 2α,3β-$(OAc)_2$ and 2α,3α-$(OAc)_2$ are close, their configurations can be differentiated between by using the C-3 proton's position (4.75 ppm of H-3α < 4.96 ppm of H-3β) and its coupling constant (10.5 Hz of H-3α > 3 Hz of H-3β).

* Part 4 in the series 'Constituents of the Labiatae Plants' For Part 3 see ref. [1].

‡ Examples of abbreviation. 2β,3α-$(OH)_2$ or 2α,3α,23-$(OAc)_3$ = triterpenes having OH or OAc functions at positions, and in the configurations, indicated

Table 1 ^{1}H NMR signals of methyl 2,3-dihydroxy urs-12-en-28-oates and their diacetates

Assignments	2β,3α-(OH)₂*	2β,3α-(OAc)₂*	2β,3β-(OH)₂	2β,3β-(OAc)₂*	2α,3β-(OH)₂	2α,3β-(OAc)₂	2α,3α-(OH)₂	2α,3α-(OAc)₂
2-H	3.75 *ddd* (17, 10, 2)	4 95 *dd* (13, 6 5)	4 08 *ddd* (4, 4, 3)	5.31 *ddd* (4, 4, 3)	3 68 *ddd* (11, 10, 4 5)	5 10 *ddd* (11, 10 5, 4 5)	4 00 *ddd* (12, 4 5, 3)	5 23 *ddd* (12, 4 5, 3)
3-H	3 63 *d* (10)	5 03 *d* (6 5)	3.20 *d* (4)	4 61 *d* (4)	2.99 *d* (10)	4 75 *d* (10 5)	3 43 *d* (3)	4 96 *d* (3)
12-H	5 27 *t* (3 5)	5.25 *t* (3 5)	5 25 *t* (3 5)	5.24 *t* (3.5)	5 24 *t* (3 5)	5 23 *t* (3 5)	5 25 *t* (3 5)	5 25 *t* (3 5)
18-H	2 23 *d* (11)	2 23 *d* (11)	2 22 *d* (11)	2 22 *d* (11)	2 22 *d* (11)	2 23 *d* (11)	2 23 *d* (11)	2 24 *d* (11)
23-H_3	0 90 *s*	1.00 s	1 00 s	0 89 s	1 02 s	0 89 s	1 02 s	0 87 s
24-H_3	1 00 *s*	0 91 *s*	0 99 *s*	1 04 *s*	0 81 *s*	0 90 *s*	0.86 *s*	0 98 *s*
25-H_3	1 09 *s*	1 14 *s*	1 23 *s*	1.19 *s*	0.98 *s*	1.06 *s*	0 97 *s*	1 03 *s*
26-H_3	0 73 *s*	0.75 *s*	0 75 *s*	0.76 *s*	0.73 *s*	0 74 *s*	0 73 *s*	0.74 *s*
27-H_3	1 07 *s*	1 10 s	1 06 s	1 06 *s*	1.07 *s*	1 06 *s*	1 08 *s*	1 11 *s*
29-H_3	0 85 *d* (6)	0 85 *d* (6)	0 84 *d* (6)	0 85 *d* (6)	0.85 *d* (6)	0.84 *d* (6)	0.85 *d* (6)	0 85 *d* (6)
30-H_3	9 93 *d* (6)	0 94 *d* (6)	0 93 *d* (6)	0 94 *d* (6)	0.93 *d* (6)	0 94 *d* (6)	0.94 *d* (6)	0 94 *d* (6)
OMe	3.59 *s*	3 60 *s*	3 59 *s*	3 60 *s*	3 59 *s*	3 60 *s*	3 60 *s*	3 60 s
OAc		2.01 *s*		2.02 *s*		1 97 *s*		1 95 *s*
		2 06 *s*		2 04 *s*		2 05 *s*		2 11 *s*

*Measured at 300 MHz, the rest at 400 MHz.

†Values in parentheses are coupling constants in Hz

The differences in ^{13}C NMR spectral data at C $-8,11 \sim 22$ and $26 \sim 30$ among eight compounds are less than 0.5 ppm The C-4 and C-10 in the group with 2α-OH and 2α-OAc appear at higher fields than in that with 2β-OH and 2β-OAc, and C-9 shows the reverse relation, whereas the C-5 in the group with 3β-OH and -OAc absorbs at higher field than in that with 3α-OH and -OAc, and the C-1 also behaves in the similar manner except for 2β,3α-$(OH)_2$ and -$(OAc)_2$ By the values of the C-6,7,23,24 and 25,2β,3α-$(OH)_2$ and -$(OAc)_2$ can be distinguished from the other three pairs. Moreover, these distorted features of both A rings seem to be slightly alterable by the considerable differences of each spectral datum.

Pentacyclic triterpenes with 2,3,23- and 2,3,24-triacetoxy substituents

The assignment of the hydroxy methine and methylene protons at C-2, C-3 and C-23 or 24 in trihydroxy derivatives is often difficult because of their overlapping resonances, while those in the corresponding acetates could be assigned with comparative ease. As shown in Table 3, the H-2, H-3 and H-12 signals are found in the following range, δ 5.48 ppm > H-2α > 5.30 ppm > H-2β > 5.16 ppm, δ 5 35 ppm > H-3β > 5.12 ppm, δ 5.09 ppm > H-3α > 4 86 ppm and δ 5.22 ppm > H-12 > 5.58 ppm. The general order among these three protons is H-12, H-2 and H-3 from the lower-field side, except for H-3, H-12 and H-2 in 2α,3α,24-$(OAc)_3$ (H-12, H-3 and H-2 in compound **17**) and H-2, H-12 and H-3 in 2β,3β,23-$(OAc)_3$. Furthermore, the differences between H-2 and H-3 of triacetates and those of diacetates are shown in Table 4, which indicate that the shifts are effected by the 23- or 24-acetoxy function.

The $\Delta\delta_{2-3}$ values are from 0.51 ppm to 0.43 in 2β,3β,23-$(OAc)_3$ > 0.33 to 0.32 in 2α,3β,24-$(OAc)_3$ > 0.22 to 0.08 in 2α,3β,23-$(OAc)_3$ > 0 08 to 0.04 in 2α,3α,23-$(OAc)_3$ > −0.1 to −0.15 in 2α,3α,24-$(OAc)_3$. Though the difference between $\Delta\delta_{2-3}$ values in 2α,3β,23- and 2α,3α,23-$(OAc)_3$ is very small, their configurations are clearly distinguished by using the C-3 proton's location (β > δ 5.1 ppm > α) and their *J*-values (α, *ca* 4 and 10 Hz > β; 3 Hz).

Applied examples for some triterpenes

The assignments of 2β,3β- and 2α,3α-$(OH)_2$ in oleanene- and ursene-2,3-diols synthesized by Brieskorn and Krause [37] were the same as assigned by Djerassi *et al* [38] and Tschesche *et al.* [39], but they should be revised as reported by Cheng and Yan [13] and Biessels *et al.* [15] Hence '2β-hydroxyoleanolsäure' reported as one of components isolated from *Rosmarinus officinalis* (Labiatae) [40] and *Melissa officinalis* (Labiatae) [37] must be revised to be 2α,3α-dihydroxyolean-12-en-28-oic acid (**24**) (3-epimaslinic acid is also supported by following datum; doublet at δ 4 95 [40] = H-3β, as shown in Table 1), which was later isolated from the other four Labiatae plants [22, 29, 30, 41, 42].

Roxburic acid isolated from *Rosa roxburghii* (Rosaceae) was assigned to be 2β,3α,7β,19α-tetrahydroxyurs-12-en-28-oic acid by Liang [19] from the application of the methyl group data calculated by Takahashi *et al.* [14]. However, this application led to make a mistake, since in the assignment of C-24 and C-25 methyl groups the effect of 7β-OAc was not considered. From the data of $\Delta\delta_{2-3} = 0.16 \sim 0\,18$ ppm and $J_3 = 3$ Hz, its hydroxy configuration in A ring should be 2α,3α-$(OH)_2$, namely 2α,3α,7β,19α-tetrahydroxyurs-12-en-28-oic acid (**25**) The

Table 2. ^{13}C NMR signals of methyl 2,3-dihydroxy urs-12-en-28-oates and their diacetates

C	2β,3α-(OH)₂*	2β,3α-(OAc)₂*	2β,3β-(OH)₂	2β,3β-(OAc)₂*	2α,3β-(OH)₂	2α,3β-(OAc)₂	2α,3α-(OH)₂	2α,3α-(OAc)₂
1	47.2	41.7	44.3	42.1	46.6	44.1	41.9	39.0
2	68 9	70.5	71.0	69.6	68.9	70.1	66 5	68 3
3	78.2	76.1	78.4	78.0	83.9	80.7	78 9	77.1
4	37.5	36.9	38.0	37.3	39.2	39 3	38 3	38.4
5	51 0	50.5	55.2	55.2	55.3	54 9	48 1	49 6
6	19 8	18.8	18.1	18.0	18.3	18.2	17.9	17.8
7	32.4	32.5	32.9	32.9	32.8	32.8	32 7	32.6
8	39.7	39.6	39.6	39.6	39.5	39.5	39.6	39.7
9	48.2	47.9	47.9	48 0	47.5	47.5	47.2	47.4
10	37.3	36 6	36.6	36.7	38.2	38.1	38.2	38.1
11	23.3	23.3	23.4	23.4	23.3	23.4	23.2	23.3
12	125.6	125.4	125.6	125.3	125.3	125 1	125 3	125 2
13	138.1	138.2	138.1	138.4	138.2	138 3	138 2	138 2
14	42.2	42.2	42.1	42.2	42.2	42.1	42.1	42.1
15	27 9	27.9	27 9	27.9	28.0	28.0	27.9	28.0
16	24 2	24.2	24.2	24.2	24.2	24 2	24 2	24.2
17	48.2	48.1	48.1	48.1	48.1	48 1	48 1	48.0
18	53.0	52 9	52.9	52.8	52.8	52 8	52 8	52.8
19	39.0	39 0	39.0	39.0	39.0	39 0	39.0	39.0
20	38.8	38.9	38.8	38.9	38.8	38 9	38.8	38.9
21	30.7	30.6	30.6	30.6	30.6	30.6	30.6	30.6
22	36.6	36.6	36.6	36.6	36.6	36.6	36.6	36.6
23	23 8	22.2	29.7	29.1	28.6	28.5	28.5	27 7
24	23 2	26.6	17.3	17.7	16.8	17.7	21 8	21 6
25	21.1	18.5	16.4	16.1	16.7	16 5	16 4	16 3
26	16 7	16.7	16.9	17.0	16.9	16.9	16.9	16.9
27	23.6	23.7	23.6	23.6	23.6	23 6	23 7	23 7
28	178.1	178.0	178.0	178.0	178.0	178.0	178.1	178.0
29	17 0	17 0	17.0	17.0	17.0	17.0	17 0	17.0
30	21.1	21.1	21.1	21.1	21.2	21 2	21 2	21.2
CO_2Me	51.5	51.4	51.4	51.4	51.4	51.5	51.5	51.5
$MeCO_2$		170.3		170.7		170 8		170.7
		170.0		170.3		170 5		170 4
$MeCO_2$		21.3		21.3		21.1		21.1
		21.0		20.9		20 9		21 0

*Measured at 75.2 MHz; the rest at 100 MHz.

Δ^{12} ursene triterpene having 2α,3α-dihydroxy groups had been isolated from *Prunus* spp. of the same family [15].

Myrianthic acid isolated from *Myrianthus arboreus* (Urticaceae) by Ojinnaka *et al.* [18] was identified as 2α,3α,19α,24-tetrahydroxyurs-12-en-28-oic acid because the calculated and observed values for the chemical shifts of the angular methyl groups were in accordance with each other. However, we entertained doubts about the assignment of H-3 (δ 4.94 ppm), and the calculated values of H_3-23 (δ 1.02) and H_3-24 (0.92). The spectral chart sent by Ojinnaka indicated that its configuration is 2α,3α,23-$(OAc)_3$. On acetylation, the H_3-24 methyl singlet in 2α,3α,23-$(OAc)_3$ (δ1.07 [22] and 1.09 in **11**) shifts to lower-field than that of H_3-23 in 2α,3α,24-$(OAc)_3$ (δ0.92 to 0.98 [22, 27, 30]). Consequently, this triterpene agrees with 2α,3α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**12a**) isolated from *Coleus amboinicus* (Labiatae) [29].

The aglycone of the saponin isolated from *Polygala japonica* (Polygalaceae) by Fang *et al.* was determined as 2α,3α,24-trihydroxyolean-12-en-28-oic acid (**13a**) [43], which was also isolated from *Prunella vulgaris* (Labiatae) by us [22]. However, the spectral data for each compound is too different for them to be identical. The former

^1H NMR spectral data were recorded that H-2β is δ 5.39 (*m*, $W_{1/2}$ = 16 Hz), H-3β, 4.91 (*d*, *J* = 4 Hz), H_2-24, 3.64 and 3.81 (2*d*, *J* = each 12 Hz) and H-12, 5.33 ppm(s), whereas the latter data is shown in the part of compound **13** in Table 3. The former acetoxy configurations should be 2β,3β,23-$(OAc)_3$ from the following data, the position order = H-2, H-12 and H-3, $\Delta\delta_{2-3}$ = 0.48 ppm, J_3 = 4 Hz and methylene signals < δ3.9. Furthermore, the ^{13}C NMR spectral data can be also explained by changing data as indicated in Table 5, which were performed in comparison with the published data of platycodigenin derivative (**22b**) [44] and phytolagenin derivative (**22c**) [45]. Hence this agylcone is identical with bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid) (**22a**) obtained from *Castanospermum australe* (Leguminosae) [46]. On chemotaxonomic grounds, A ring in *Polygala* sapogenins was occupied by 2β,3β-glycol system.

The triterpene isolated from *Nepeta hindostana* (Labiatae) was elucidated as 2β,3α,23-trihydroxyurs-12-en-28-oic acid [47]. We are interested in the rarity of its substituents but at the same time have doubts about the assignment of its protons alpha to hydroxy groups, in particular H_2-23 (δ2.93, AB*q*, *J* = 10 Hz) and H-2 (δ3.68,

Table 3 Chemical shifts of H-12, and methine and methylene protons of carbons carrying acetoxy groups in 2,3,23- and 2,3,24-triacetoxylated triterpenes

Compound	Basic skeletons	Other functions	H-2	H-3	$\Delta\delta_{2-3}$	H_2-23 or 24		$\Delta\delta_{B-A}$	H-12	Ref
2α,3β,23-(OAc)$_3$			H-2β	H-3α		H_A-23 and H_B-23				
1	II		5.16 *m*	5 02 *d*	0 14	3 54 *d*	3.86 *d*	0.32	5.22 *t*	[22]
			(22)	(10)		(12)	(12)		(4)	
2	II		5 17 *dd*	5 09 *d*	0 08	3.59 *d*	3 88 *d*	0.29	5 28 *t*	[23]
			(10, 4)	(10)		(12)	(12)		(3.8)	
3	I	19α-OH 28-COOH	5 20 *m*	5.05 *d*	0.15	3 58 *d*	3 83 *d*	0 25	5.41 *m*	[24]
				(10)		(12)	(12)			
4	II	19α-OH 28-COOH	5 20 *m*	5.05 *d*	0 15	3.57 *d*	3 84 *d*	0 27	5 33 *m*	[24]
				(10)		(12)	(12)			
5	II	19α-OH	5 17 *ddd*	5.08 *d*	0 09	3 59 *d*	3.85 *d*	0.26	5.35 *t*	[25]
			(9 9, 9.9, 4 6)	(10 5)		(11.9)	(11 9)		(3.8)	
6	II	19α-OH 7α-OAc	5.24 *t*	5 02 *d*	0 22	3.74 *d*	3.95 *d*	0 21	5 38 *t*	[26]
		28-COOH	(10, 4)	(10)		(12)	(12)		(4)	
2α,3β,24-(OAc)$_3$						H_2-24				
7	II		5 18 *td*	4 86 *d*	0 32	4 20 *s*			5 25 *brt*	[27]
			(10 5, 4 5)	(10 5)					(3.5)	
8	I	19α-OH	5 18 *ddd*	4 85 *d*	0 33	4 22 *s*			5 45 *m*	[28]
			(11, 10.4)	(11)						
9	II	19α-OH 28-COOH	5 17 *td*	4 85 *d*	0.31	4.22 *s*			5 34 *t*	[26]
			(10, 4)	(10)					(4)	
2α,3α,23-(OAc)$_3$			H-2β	H-3β		H_A-23 and H_B-23				
10	I		5 18 *m*	5.12 *d*	0 06	3 71 *d*	4 04 *d*	0 33	5 25 *t*	[22]
			(22)	(3)		(12)	(12)		(4)	
11	II		5 22 *m*	5 17 *d*	0 05	3 74 *d*	4.07 *d*	0.33	5 24 *t*	
			(22)	(3)		(11)	(11)		(3.5)	
12	II	19α-OH	5 26 *m*	5.18 *d*	0 08	3 88 *q*			5 35 *m*	[29]
			(9)	(3 5)						

2α,3α,24-(OAc)$_3$						H_A-24 and H_B-24				
13	I		5.18 *ddd* (12, 5, 3)	5.33 *d* (3)	−0.15	4.04 *d* (12)	4.18 *d* (12)	0.14	5.30 *t* (3 5)	[30]
14†	II		5.17 *ddd* (12, 5, 3)	5.31 *d* (3)	−0.14	4.03 *d* (12)	4.17 *d* (12)	0.14	5.24 *t* (3.5)	[30]
15	II	$\Delta^{20(30)}$	5.18 *ddd* (12, 5, 3)	5.31 *d* (3)	−0.13	4.03 *d* (12)	4.17 *d* (12)	0.14	5.29 *t* (3.5)	[30]
16	I	$\Delta^{11,13(18)}$	5.25 *ddd* (12, 5, 3)	5.35 *d* (3)	−0.10	4.06 *d* (12)	4.17 *d* (12)	0.11	5.58 *d* (11)	[30]
17	II	19α-OH	5.20 *m* (18)	5.34 *d* (3)	−0.14	4.06 *d* (12)	4.21 *d* (12)	0 15	5.38 *t* (3)	[23]
18	II		—	5.32 *brs*		4.02 *d* (12)	4.22 *d* (12)	0.20	—	[31]
2β,3β,23-(OAc)$_3$			H-2α	H-3α		H_A-23 and H_B-23				
19	I	30-COOMe	5.38 *brs*	4.95 *d* (4)	0.43	3.87 *q* (each 10.9)			5 35 *brs*	[32]
20	I	$\Delta^{5(6)}$	5.32-5.48‡	5.02 *d* (4)	0.30-0.46	3.81 *d* (12)	4.22 *d* (12)	0.42	‡	[33]
21	I	6β-OH	5.24-5.46‡	4.86 *d* (4)	0.38−0.6	3.87 (*ABq* 13)	3.91		‡	[33]
22	I		5.4 *m*	4.95 *d* (4)	0.45	3.8 *q*			5.4 *m*	[34]
23	I	16β-OAc 21→β28-lactone	5.42 *brs*	4.91 *d* (3.9)	0.51	3 72 *d* (12)	3.85 (12)	0.13	5.41 *t* (3.5)	[35]

*The figures in parenthesis are coupling constants in Hz except for $W_{1/2}$ values in *m*.

†We regret that having isolated this compound from *Hedyotis lawsoniae* [27] is misquoted to ref. [30].

—not reported.

‡H-2 and H-12 were overlapped.

§In a recent short report [36] on arboreic acid (2β, 3β, 24-trihydroxyolean-12-en-28-oic acid), the NMR spectral data of its acetate is not recorded

Table 4 The effect of 23-OAc or 24-OAc on H-2 and H-3 ($\Delta\delta = \delta_{2,3,23\ or\ 24-OAc} - \delta_{2,3-OAc}$, ppm)

Compounds		23-OAc	24-OAc
2α,3β,23 or 24-$(OAc)_3$	H-2β	+0 06 to 0 14	+0 07 to 0 08
	H-3α	+0.27 to 0 34	+0 10 to 0 11
2α,3α,23 or 24-$(OAc)_3$	H-2β	−0 05 to +0.03	−0 06 to +0 02
	H-3β	+0 16 to 0 18	+0 36 to 0 39
2β,3β,23-$(OAc)_3$	H-2α	+0 07 to 0.11	
	H-3α	+0 25 to 0 41	

Table 5 ^{13}C chemical shifts of compounds **13** and **13b** and our assignment for **22** partially exchanging **13b** by comparison with **22b** and **22c**

C	13	13b	22	22b	22c
1	38 5	47 9*†	41 6 (C-14)‡	41 6	41.7
2	67 7	65.7*	69.7 (C-24)	69 6	69.6
3	72 4	71 9	71 9	72 0	72 0
4	41 6	40 9	40.9	40 1	40 1
5	50 1	48 0*	48 0	48 2	47 4
6	18 0	17.7	17 7	17 7	17 7
7	32.5	32 9	32.9	32 3	32 6
8	39 3	39 5	39 5	39 4	39 6
9	47 5	47 7	47 7	47 8	47 8
10	38.1	39.3*	39.3	36 7	36 7
11	23 5	22 6	23 5 (C-16)	23 0	23.4
12	121 8	122 3	122 3	123 1	123 4
13	143.8	143 6	143 6	143 1	142 1
14	42 0	41 6	41 6	41.6	41 2
15	27 5	27 6	27 6	27 5	32.1
16	22 9	23 5	22 6 (C-11)	23 4	76.2
17	46 5	46.4	46.4	45 9	47.6
18	41.1	41 1	41 1	42 3	40.4
19	45 8	45 7	45 7	42 0	46 1
20	30 6	30 6	30 6	43 7	30 4
21	33 7	36 6*	36 6	30.3	35 1
22	32 2	32 3	32 3	33 5	31 0
23	22 1	16 4*	65 7 (C-2)	65.5	65 5
24	66 1	69 7*	13 7 (C-25)	13 7	13 9
25	16 6	13.7*	16 4 (C-23)	16 5	16 6
26	16 7	17 2	17.2	17 2	17 0
27	25 9	25 8	25 8	25 8	26 3
28	178 1	184*	184	182 8	176.0
29	33 0	33 8	33 8	28 3	33 2
30	23 5	23 5	23 5	176 9	24.2
CO_2Me	51 5			51 7	51 5
$MeCO_2$	170.1	169 5	169 5	170 0	169 8
	170 3	170 0	170.0	170.3	170 0
	171 1	170 0	170 0	170 7	170 3
					170 7
$Me\,CO_2$	20 8	20.7	20 7	20 7	20 8
	20 9	21 1	21 1	20.8	20 8
	21 1	22 9*	22 9	21 2	21 2
					22.0

*The differences of these figures in comparison with compound **13** are more than 1.2 ppm

†Only unassigned peak in compound **22**

‡The C-figure in brackets indicates carbon of **13b** that had been assigned in ref. [43]

|The differences of these data in comparison with those of **22** are less than 0 8 ppm

d, *J* = 3 Hz) On re-examination of the NMR spectral data of its methyl ester sent by Ahmad, his and co-worker's assignments were found to be in error because of the low resolution of its spectra. Its structure can be assigned as 2α,3α,23-trihydroxyurs-12-en-28-oic acid (**11a**) (named as esclentic acid), already isolated from *Diplazium esculentum* (Athyriaceae) [48], *Hedyotis lawsoniae* (Rubiaceae) [27], and now from the leaves and stems of *Prunella vulgaris* (see Experimental)

In conclusion, the characteristics of proton signals on the acetoxy (or hydroxy)-bearing carbons, i.e. their position (its relationship between them), splitting pattern and coupling constant (or half-height width), give valuable information about the position and configuration of hydroxy groups. Thus, when the spectral data of high-resolution NMR are not available, it is at least necessary to measure ^1H NMR spectra of the acetyl derivative

EXPERIMENTAL

NMR measurements ^1H and ^{13}C NMR spectral data were recorded as δ values at room temp in $CDCl_3$ on a Varian XL-300 or 400, which were assigned by DEPT pulse sequence or by comparative studies of ^1H-^1H and ^1H-^{13}C 2D COSY spectral data.

Condition of HPLC Senshu gel 5C-18-H, 10 mm × 30 cm, at room temp

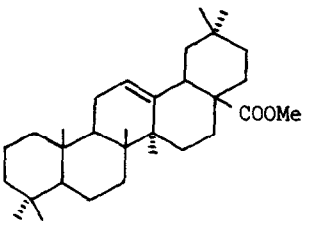
Methyl 2α,3α-dihydroxyurs-12-en-28-oate [2α,3α-$(OH)_2$] *and methyl 2β,3β-dihydroxyurs-12-en-28-oate* [2β,3β-$(OH)_2$] These compounds were prepared by oxidation (8 days) of methyl urs-2,12-dien-28-oate (310 mg) [13, 38] with OsO_4 (650 mg)/dioxane (12 ml) After removal of the catalyst, the filtrate was evapd and the residue purified by silica gel CC and HPLC (column Whatmann Partisil 5 ODS-3, 10 mm × 25 cm, mobile phase, MeOH–H_2O 17 3) to yield 2α,3α-$(OH)_2$ (R_t 19 1 min, 31 6 mg), 2β,3β-$(OH)_2$ (25.0 min, 27 9 mg) and byproduct (17.8 min, 7 0 mg) For ^1H NMR see Table 1, and for ^{13}C NMR see Table 2

Methyl 2β,3α-dihydroxyurs-12-en-28-oate [2β,3α-$(OH)_2$] By a similar procedure to ref [49], methyl urs-2,12-dien-28-oate (50 mg) in CH_2Cl_2 (2 ml) was added to *m*-chloroperbenzoic acid (100 mg) in CH_2Cl_2 (2 ml) The mixture was left at room temp for 2 hr, poured into ice-water, and extracted into Et_2O

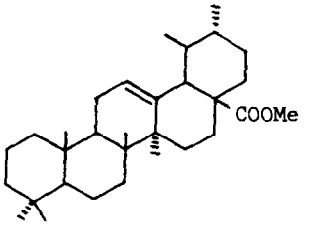
The extract in Me_2CO (2 ml) and $HClO_4$ (0 1 ml, 7%) was again left at room temp for 16 hr and then poured into H_2O. The residue isolated by Et_2O was purified by HPLC (mobile phase, MeOH–H_2O (19 1), flow rate, 2 6 ml/min) to yield main two fractions, 2β,3α-$(OH)_2$ (23 5 min, 17 6 mg) and byproduct (21.0 min, 5 9 mg)

Methyl-2α-hydroxyursolate [2α,3β-$(OH)_2$] The fraction containing 2α,3β-$(OH)_2$ and methyl maslinate [22] was separated by HPLC (mobile phase, MeOH–H_2O 97 3, flow rate, 2 5 ml/min) to afford 2α,3β-$(OH)_2$ (19 3 min, 10 5 mg) and methyl maslinate (18 2 min, 7 0 mg)

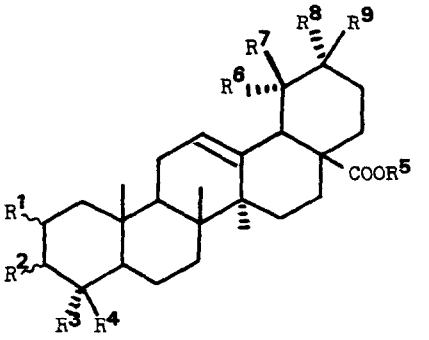
Methyl 2α,3α,23-trihydroxyurs-12-en-28-oate (**11b**) The filtrate left removing crystals of methyl 2α,3α,23-trihydroxyolean-12-en-28-oate (**10b**) [22] was again separated by HPLC (mobile phase, MeOH–H_2O 9 1, flow rate, 2 5 ml) to give **10b** (9 8 min, 7.2 mg) and **11b** (11 6 min, 4 0 mg) ^1H NMR of **11b** δ 0 72 (*s*, H_3-26), 0.75 (*s*, H_3-24), 0 86 (*d*, *J* = 6 Hz, H_3-29), 0 94 (*d*, *J* = 6 Hz, H_3-30), 0 99 (*s*, H_3-25), 1 10 (*s*, H_3-27), 2 23 (*d*, *J* = 11 Hz, H-18), 3 47, 3 53 (2*d*, *J* = 12 Hz, H_A-23 and H_B-23), 3 60 (*s*, CO_2Me), 3 67 (*d*, *J* = 3 Hz, H-3β), 3 98 (*ddd*, *J* = 11 5, 5, 3 Hz H-2β), 5 26 (*t*, *J* = 3 5 Hz, H-12) This signal pattern was essentially identical with that obtained by Ahmad ^{13}C NMR δ 41 6 (C-1), 66 6 (C-2), 78 6 (C-3), 41 1 (C-4), 42 1 (C-5), 17 8 (C-6), 32 4 (C-7), 39 6 (C-8), 47 9



Methyl olean-12-en-28-oate (**I**)



Methyl urs-12-en-28-oate (**II**)



	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	other function
11	αOAc	αOAc	CH_2OAc	Me	Me	H	Me	Me	H	
11a	αOH	αOH	CH_2OH	Me	H	H	Me	Me	H	
11b	αOH	αOH	CH_2OH	Me	Me	H	Me	Me	H	
12a	αOH	αOH	CH_2OH	Me	H	OH	Me	Me	H	
13	αOAc	αOAc	Me	CH_2OAc	Me	H	H	Me	Me	
13a	αOH	αOH	Me	CH_2OH	H	H	H	Me	Me	
13b	αOAc	αOAc	Me	CH_2OAc	H	H	H	Me	Me	
22	βOAc	βOAc	CH_2OAc	Me	H	H	H	Me	Me	
22a	βOH	βOH	CH_2OH	Me	H	H	H	Me	Me	
22b	βOAc	βOAc	CH_2OAc	Me	Me	H	H	Me	CO_2Me	
22c	βOAc	βOAc	CH_2OAc	Me	Me	H	H	Me	Me	16βOAc
24	αOH	αOH	Me	Me	H	H	H	Me	Me	
25	αOH	αOH	Me	Me	H	OH	Me	Me	H	7βOH

(C-9), 37.9 (C-10), 23.3 (C-11), 125.2 (C-12), 138.4 (C-13), 42.1 (C-14), 28.0 (C-15), 24.2 (C-16), 48 1 (C-17), 52.9 (C-18), 39 0 (C-19), 38 9 (C-20), 30 6 (C-21), 36 6 (C-22), 71.3 (C-23), 17.5 (C-24), 16.8 (C-25), 16.9 (C-26), 23.7 (C-27), 178 1 (C-28), 17 0 (C-29), 21.2 (C-30), 51.5 (CO_2Me). These assignments were based on the published spectral data of methyl 2α,3α,23-trihydroxyolean-12-en-28-oate [22] and methyl 2α,3α,24-trihydroxyurs-12-en-28-oate [30]. (These results were almost consistent with our re-assignments of data provided by Ahmad)

Triacetate of **11b** (**11**). ^{1}H NMR: δ 0.75 (*s*, H_3-26), 0 85 (*d*, J = 6 Hz, H_3-29), 0 94 (*d*, J = 6 Hz, H_3-30), 1.09 (*s*, H_3-24), 1.11 (*s*, H_3-25) 1 12 (*s*, H_3-27), 2.24 (*d*, J = 12 Hz, H-18), 3.60 (*s*, CO_2Me), H-2, H-3, H-12, H_2-23 (see Table 3). ^{13}C NMR: δ 38.8 (C-1), 67.8 (C-2), 77.2 (C-3), 41.0 (C-4), 42.0 (C-5), 18.4 (C-5), 18.4 (C-6), 32.3 (C-7), 39.4 (C-8), 47.4 (C-9), 38.4 (C-10) 23.2 (C-11), 125.0 (C-12), 138.3 (C-13), 42 1 (C-14), 27.9 (C-15), 24.1 (C-16), 48 0 (C-17), 52.8 (C-18), 39.0 (C-19), 39.0 (C-20), 30.6 (C-21), 36.6 (C-22), 71.8 (C-23), 17.2 (C-24), 16.5 (C-25), 16.9 (C-26), 23.7 (C-27), 178.0 (C-28), 16.9 (C-29), 21.2 (C-30), 51 5 (CO_2Me), 170.1, 170 4, 171.4 ($MeCO_2$), 20 9, 21 0, 21.0 ($MeCO_2$).

Acknowledgements—The authors express their deep gratitude to Dr V. U. Ahmad, University of Karachi, for ^{1}H and ^{13}C NMR spectral data and Dr C M. Ojinnaka, University of Port Harcourt, for ^{1}H NMR spectral data. We are indebted to members of the Analytical Centre of Kitasato University for 300 and 400 MHz NMR spectra.

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