and 912; ^{1}H NMR (100 MHz, $CDCl_3$): δ 0.84 (3H, *s*), 0.92 (3H, *s*), 0.98 (3, *d*, $J = 6$ Hz), 3.74 and 3.90 (1H, each, *d*, $J = 10$ Hz), 6.20 (1H, *d*, $J = 2$ Hz) and 7.31 (1H, *d*, $J = 2$ Hz); MS *m/z* (rel. int.): 344 $[M]^+$ (35), 329 (5), 269 (10), 108 (100) and 55 (15).

Tosylation of **3**. Alcohol **3** (50 mg), in dry pyridine (1 ml), was treated with TsCl (90 mg) at room temp. for 1 day. The reaction mixture was worked-up in the usual way, and the crude tosylate **6** purified by preparative silica gel TLC (hexane–EtOAc, 3:1) to afford pure **6** (39 mg). ^{1}H NMR (100 MHz, $CDCl_3$): δ 0.80 (3H, *s*), 0.84 (3H, *s*), 0.98 (3H, *d*, $J = 6$ Hz), 2.50 (3H, *s*), 3.82 and 4.16 (1H, each, *d*, $J = 9$ Hz), 6.16 (1H, *d*, $J = 3$ Hz), 7.15 (1H, *d*, $J = 3$ Hz), 7.36 (2H, *d*, $J = 8$ Hz) and 7.80 (2H, *d*, $J = 8$ Hz).

$LiAlH_4$ *reduction of* **6**. Tosylate **6** (35 mg), in dry THF (2 ml), was refluxed with an excess of $LiAlH_4$ for 15 hr. The product was extracted and purified (prep silica gel TLC hexane–EtOAc, 9:1) to yield pure **5** (22 mg) which was identical (IR, UV, MS and ^{1}H NMR) with an authentic sample.

Acknowledgements—The authors are grateful to Dr Edilberto Rocha Silveira, Universidade Federal do Ceará, for hydrocarbon **5**, and Dr Pierre Albrecht, ULP, Strasbourg, France, for HRMS and as well as Nucleo de Pesquisa de Produtos Naturais (NPPN) for NMR data. This work was financially supported by grants from INPA-MCT, CNPq and CEPG-UFRJ.

REFERENCES

1. Sullivan, G. J. (1982) *J. Food Chem.* **30**, 609.
2. Hayashi, T. and Thomson, R. H. (1974) *Phytochemistry* **13**, 1943.
3. Nakano, T. Alonso, J., Grillet, R. and Martin. A. (1979) *J. Chem. Soc. Perkin Trans. I* **9**, 2107.
4. King, F. E., Godson, D. H. and King, T. J. (1955) *J. Chem. Soc.* 1117.
5. Polonsky, J. Baskevitch, Z., Cagnoli-Bellavita, N., Ceccherelli, P., Buckwalter, B. L. and Wenkert, E. (1972) *J. Am. Chem. Soc.* **94**, 4369.
6. Orsini, F., Pelizzoni, F. and Ricca G. (1981) *Org. Magn. Reson.* **17**, 163.

Phytochemistry, Vol. 28, No. 2, pp. 644–647, 1989.
Printed in Great Britain.

0031-9422/89 $3.00 + 0.00
Pergamon Press plc.

TRITERPENOID SAPONINS FROM PLANTS OF ARALIACEAE*

S. K. SRIVASTAVA* and D. C. JAIN

Central Institute of Medicinal and Aromatic Plants, Lucknow, India

(*Received in revised form* 29 *July* 1988)

Key Word Index—*Schefflera impressa*; *Macropanax disperum*; Araliaceae; triterpenoids; triterpenoid glycosides.

Abstract—Two new triterpenoid saponins were isolated from the bark and stem of *Schefflera impressa* C. B. Clarke and characterized as 3β,23-dihydroxy-urs-12-en-28-oic acid-3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester and 4-epihederagenin-3-*O*-β-D-glucuronopyranoside-6′-*O*-methyl ester alongwith oleanolic acid; hederagenin; 23-hydroxy ursolic acid and hederagenin-3-*O*-β-D-glucuronopyranoside-6′-*O*-methyl ester. While leaves of *Macropanax disperum* Blume led to the isolation of 3β-hydroxy-olean-12-en-28-*O*-β-D-glucopyranoside; oleanolic acid and hederagenin.

INTRODUCTION

Schefflera impressa C. B. Clarke (Napalese name 'Balu Chinia') and *Macropanax disperum* Blume are evergreen woody trees of the family Araliaceae, which are distributed in the Himalayas from Kumaun to Bhutan. No work has been done on the constituents of these plants, however. As a part of our studies on Indian medicinal plants for their biological active saponins [1] these plants have now been examined. The present communication describes the isolation and structure elucidation of two new triterpenoid glycosides.

RESULTS AND DISCUSSION

The stem and bark of *S. impressa* and leaves of *M. disperum* were extracted with methanol. The water soluble portion of the methanol extract was successively extracted with hexane, benzene, chloroform, ethyl acetate and butanol. The butanol-soluble fraction of *S. impressa* was subjected to repeated vacuum liquid chromatography (VLC) [2] over TLC silica gel to give sapogenin SI-A (**1**, **2**) saponins SI-B (**2a**) and C (**3**, **4**). On TLC SI-A, B and C showed a single spot and could not be resolved in several solvent systems.

Isomeric mixtures of olean-12-ene and urs-12-enes as well as hederagenin and its isomer 4-epihederagenin [3] have frequently been isolated from the plant kingdom. Separation of these triterpenoids is a challenging problem that remain unsolved. However ^{13}C NMR spectroscopy permits their identification [3–5].

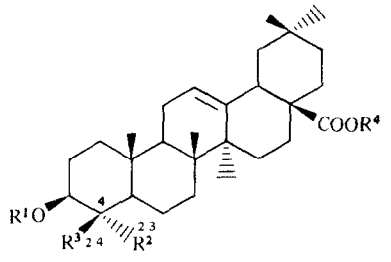
Sapogenin SI-A obtained from *S. impressa* contained the triterpenes **1** and **2**. On the basis of ^{13}C NMR analysis of SI-A, they were identified as hederagenin (**1**) and 3β,23-dihydroxy-urs-12-en-28-oic acid (**2**) [4]. This is the second report of the isolation of this isomeric mixture from nature.

*CIMAP Communication No. 747.

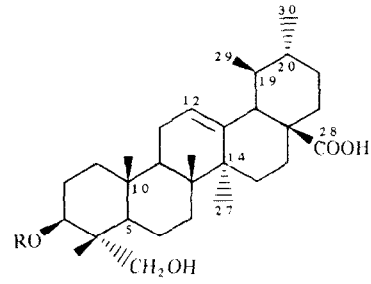
Saponin SI-B, mp 180–182°, $[\alpha]_D^{23}$ +14° (MeOH; *c* 0.5) found to be homogeneous on TLC and it gave positive Fiegal test for glycosides and exhibited broad bands at 3440 cm^{-1} (hydroxyl group), 1735 cm^{-1} (ester group), 1700 cm^{-1} (acid group) and 1630 cm^{-1} (unsaturation). Its EIMS showed ion peaks at *m/z* 472, 248, 203, 191, 189, 172 and 133, which showed that the aglycone is an amyrin derivative having a free carboxylic group at C-17 [6, 7]. Its ^{13}C NMR showed signals at δ125.0 (C_{12}) and 139.8 (C_{13}) characteristic for urs-12-ene derivative [8] which was further confirmed by its acid hydrolysis to give 23-hydroxy ursolic acid (co-TLC with SI-A, ^{13}C NMR) [4] and glucuronic acid (co-PC). The signal at δ3.40 in the ^1H NMR spectrum of SI-B indicated the presence of a carbomethoxy group in the glycone part of the saponin, which is further evidenced by the signals at δ170.8 and 51.9 in the ^{13}C NMR [9]. The presence of a signal at δ180.8 showed the presence of a free carboxylic group at C-17. Further signals at δ106.5, 75.5, 78.0, 73.2 and 77.5 assigned to C-1′, C-2′, C-3′ and C-4′ were in agreement with glucuronopyranoside 6′-*O*-methyl ester moiety [10]. The signal at 82.7 and 64.6 ppm assigned to C-3 and C-23 are δ8.5 down and 3.6 upfield than the parent compound **2**, clearly indicating the α and γ effect due to glycosidation of C-3 [11–13]. The signal at δ106.5 is characteristic of β-configuration of a sugar at C-3 [4, 13]. Thus, SI-B was

Table 1. ^{13}C NMR data for compounds **1–6**

Aglycone moiety	1	2	2a	3	4	5	6
C-1	38.7		39.0	39.0	38.5	39.4	
2	28.1		26.3	26.1	25.7	28.1	
3	73.3	74.2	82.7	82.2	92.2	78.4	78.1
4	42.6		43.8	42.7	43.7	39.5	
5	48.7		48.0	47.5	58.5	56.3	
6	18.2		18.3	18.1	20.2	19.0	
7	33.0		33.0	32.6	33.4	33.5	
8	39.6		40.0	39.8		40.0	
9	48.2		48.5	48.3		48.5	
10	37.4		37.1	36.9		37.6	
11	23.4	24.2	23.9	23.7		23.8	
12	122.2	125.5	125.0	122.3		122.5	
13	144.8	139.2	139.8	145.0		145.3	144.5
14	42.0		43.0	42.3		42.4	
15	28.5	27.3	29.0	28.0		28.5	
16	23.4	24.6	24.5	23.7		23.8	
17	46.5	47.5	47.0	46.7		47.0	
18	41.9	52.2	53.0	42.3		42.4	
19	46.5	39.2	39.0	46.7		47.0	
20	30.6		30.9	30.7		31.3	
21	34.4	37.0	33.5	34.2		34.5	
22	33.5		33.0	33.0		33.5	
23	68.2		64.6	64.4	23.4	29.0	
24	13.2	12.7	13.6	13.4	61.3	16.5	
25	16.1	15.6	16.2	16.0	15.5	15.6	
26	17.1		17.5	17.4		17.5	17.1
27	25.9	24.2	23.9	26.0		26.4	
28	182.2	179.7	180.8	180.5		180.5	176.0
29	33.5	17.1	17.5	33.0		33.5	
30	23.4	21.1	21.8	23.7		23.8	
3-*O*- sugar moiety							
C-1′			106.5	106.0	106.0		
2′			75.5	75.3	75.3		
3′			78.0	77.5	77.5		
4′			73.2	73.0	73.0		
5′			77.5	77.0	77.0		
6′			170.8	170.0	170.0		
COOMe			51.9	52.1	52.1		
28-*O*-Sugar moiety							
C-1′							95.5
2′							74.0
3′							77.1
4′							71.1
5′							79.1
6′							62.5



	R^1	R^2	R^3	R^4
1	H	CH_2OH	Me	H
3	6′-OMe-GlcUA	CH_2OH	Me	H
4	6′-OMe-GlcUA	Me	CH_2OH	H
5	H	Me	Me	H
6	H	Me	Me	Glc



2 R = H
2a R = 6′-OMe-GlcUA

identified as 3β,23-dihydroxy-urs-12-en-28-oic acid 3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester (**2a**). Saponin SI-C, mp 167–170°, $[\alpha]_D^{23}$ +4° (MeOH; *c* 0.5) gave a positive Fiegal test for glycosidic nature and exhibited broad bands at 3400 cm^{-1} (hydroxyl group), 1740 cm^{-1} (ester group), 1700 cm^{-1} (acid group) and 1640 cm^{-1} (unsaturation). Its EIMS showed ion peaks at *m/z* 472, 248, 203, 191, 189, 175 and 133, which indicated that the aglycone is the amyrin derivative having a free carboxylic group at C-17 [6, 7]. The ^{13}C NMR of SI-C showed its close resemblance with hederagenin-3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester (3) [10]. Careful analysis showed the presence of other signals having close chemical shift value to **3** which could be assigned to its 4-epiisomer (**4**) [3] by considering the difference from the corresponding hederagenin carbons, especially the C-5, C-6 and C-23 as well as those of C-24 and C-3 which are of the greatest diagnostic value. The signals at 61.8 and 92.2 showed the presence of a hydroxyl group at C-24 and glycosidation at C-3 (characteristic of 4-epihederagenin glycosides). The signals at 58.5, 20.2 and 23.4 ppm (assigned to C-5, C-6 and C-23 were in agreemnt with a triterpenoid structure containing a hydroxyl group at C-24 and glycosidation at C-3. Thus the other component of SI-C was identified as 4-epihederagenin-3-*O*-β-D-glucuronopyranoside-6′-*O*-methyl ester (**4**).

Fractionation of the butanol extract from stem and bark of *S. impressa* and leaves of *M. disperum* yielded oleanolic acid (**5**) and hederagenin (**1**) identified on the basis of their IR, ^1H, ^{13}C NMR and MS spectra [14].

Further fractionation of the butanol extract of *M. disperum* led to the isolation of saponin MD-A. Analysis of its ^{13}C NMR spectrum showed close resemblance of the aglycone signals with oleanolic acid. The signals at δ71.1, 79.1 and 62.5 assigned to C-4′, C-5′ and C-6′ were in agreement with a glucose unit. The signal at 95.5 assigned to an anomeric carbon, indicating an ester type of linkage between sugar and oleanolic acid, possible only at C-28. This was further confirmed by alkaline hydrolysis of MD-A to give oleanolic acid and glucose (CO-PC). Thus MD-A was identified as 3-hydroxyolean-12-en-28-oic acid-28-*O*-β-D-glucopyranoside (**6**) [15]. This is the second report of compound **6** from a natural source.

EXPERIMENTAL

Mps: TMS was used as int. standard in C_5D_5N solvent for ^1H and ^{13}C NMR. TLC and VLC were carried out on silica gel G using solvent system a, $CHCl_3$–MeOH–H_2O (13:7:2); b, $CHCl_3$–MeOH (19:1); c, $CHCl_3$–MeOH (9:1). Spray reagent 10% H_2SO_4; L.B. reagent; aniline hydrogen phthalate.

Extraction and isolation of compounds. Air-dried powdered stem and bark (2.3 kg) of *Schefflera impressa* and leaves of *M. disperum* (0.6 kg) collected from Darjeeling, India (voucher specimen deposited in the Botany Division of the Institute) were extracted with MeOH. The extract was concd and dissolved in H_2O. The aq. soln was fractionated with *n*-hexane, C_6H_6, $CHCl_3$, EtOAc and *n*-BuOH satd with H_2O. The BuOH fraction concd under vacuum yielded a brown viscous mass 62.8 and 30.2 g, respectively. The BuOH extract of *S. impressa* (62.8 g) was chromatographed over VLC on TLC (silica gel) (725 g) and eluted with solvent mixtures of increasing polarity.

Isolation of oleanolic acid. The EtOAc (100%) eluate (1 g) on rechromatography gave **5** (0.4 g) mp 280–282° (MeOH), [R_f 0.66 (b)]. IR ν_{max}^{KBr} cm^{-1} 3400, 2900, 2875, 1710, 1670, 1440, 1370, 1245, 1080, 1015. ^1H NMR (C_5D_5N) δ 0.75–1.15 (*s*, 7 × Me), 3.2 (1H, *t*, H-3), 5.3 (*s*, H-12). MS: *m/z* 456 $[M]^+$ 438, 423, 410, 392, 302, 248 (100), 219, 207, 203, 189, 175, 145 and 133. ^{13}C NMR, see Table 1. Isolation of sapogenin SI-A: The EtOAc–MeOH (98:2) eluate afforded a solid (1.7 g) which on repeated crystallization with MeOH furnished SI-A (0.8 g), mp 322–324°, R_f 0.5 (C). IR ν_{max}^{KBr} cm^{-1}, 3418, 2900, 2875, 1680, 1445, 1370, 1290, 1250, 1220, 1190, 1170, 1025, 1000, 975. ^1H NMR (C_5D_5N) δ 0.74, 0.75, 0.80, 0.82, 1.00, 1.05 (Me, S), 2.40 (H-18, *d*, ursene group), 3.05 (H-18, *m*, oleanene group), 3.35 (23-CH_2OH, S); 3.50 (H-3), 5.25 (H-12, *m*). MS: *m/z* 472 $[M]^+$, 454, 436, 424, 395, 381, 262, 248 (100), 223, 206, 189, 175 and 133. ^{13}C NMR: see Table 1.

Isolation of saponin SI-B. The EtOAc–MeOH (19:1) eluate afforded a solid (0.1 g), which on repeated chromatography furnished SI-B (0.035 g), mp 180–182° (MeOH), R_f 0.3(c). IR ν_{max}^{KBr} cm^{-1}, 3440, 1735, 1700, 1630. ^1H NMR (C_5D_5N) δ 0.75, 0.82, 1.06 (each *s*, *tert.* Me × 6), 2.40 (H-18, ursane group), 3.40 (3H, *s*, C 5′COOMe), 4.95 (1H, *d*, *J* = 7.0 Hz, C1′-H of glucuronate unit), 5.30 (1H, *br s*, C-12-H). ^{13}C NMR: see Table 1.

Hydrolysis of SI-B. A solution of SI-B (0.04 g) in 10% aq. HCl solution (4 ml) was heated on water bath for 5 hr. The ppt. was filtered, washed with H_2O and dried. Aglycone was identified as 23-hydroxy ursolic acid (co-TLC with SI-A and ^{13}C NMR). The filtrate was neutralized with Ag_2CO_3 solution and neutral sugar solution was checked on PC (BuOH–pyridine–H_2O 6:4:3) to identify the sugar component as glucuronic acid.

Isolation of saponin SI-C. The EtOAc–MeOH (47:3) eluate afforded a solid (0.3 g) which on repeated chromatography furnished SI-C (0.05 g), mp 167–170° (MeOH), R_f 0.29(c). IR ν_{max}^{KBr} cm^{-1}, 3400, 1740, 1700, 1640, ^1H NMR (C_5D_5N) δ 0.75; 0.84, 1.08, 1.43 (each *s*, *tert.* Me × 6); 3.55 (3H, *s*, C-5′-COOMe),

4.60 (1H, *s*, $J = 10.2$ Hz, C-4-CH_2OH), 5.0 (1H, *d*, $J = 7.0$ Hz, C-1'-H of methyl glucuronate unit), 5.25 (1H, *br s*, C-12-H).

As with BuOH extract (30.2 g) of *M. disperum* which was chromatographed separately over VLC on TLC (silica gel), (350 g) oleanolic acid (0.02 g) (**5**) and hederagenin (0.015 g) (**1**) were isolated and identified on the basis of their IR, ^{1}H NMR, ^{13}C NMR and mass spectra.

Isolation of MD-A (**6**). The $CHCl_3$–MeOH–H_2O (13:4–7:2) eluates afforded a colourless amorphous powder MD-A (100 mg) mp 244–246° (Me_2CO_3), R_f 0.42 (a) $[\alpha]_D$ +43.9 (MeOH; *c* 1). IR ν_{max}^{KBr} cm^{-1}, 3400, 2940, 1735, 1630, 1460, 1380, 1050, ^{13}C NMR: see Table 1.

Alkaline hydrolysis of saponin MD-A (**6**). Saponin MD-A (10 mg) was treated with 5% methanolic KOH and refluxed on a H_2O bath for 3 hr. After usual work-up, the soln was checked on PC which showed the presence of oleanolic acid and D-glucose.

Acknowledgement—The authors thank Dr Akhtar Husain (Director, CIMAP, Lucknow) for his keen interest and encouragement.

REFERENCES

1. Jain, D. C. (1987) *Phytochemistry* **26**, 2223.
2. Pelletier, S. W., Choksi, H. P. and Desai, H. K. (1986) *J. Nat. Prod.* **49**, 892.
3. Kizo, H. and Tomimori, T. (1982) *Chem. Pharm. Bull.* **30**, 3340.
4. Sousa, M. P., Matos, M. E. O., Machado, M. I. L., Filho, R. B., Vencato, I. and Mascarenhas, V. P. (1984) *Phytochemistry* **23**, 2589.
5. Seo, S., Tomita, Y. and Tori, K. (1975) *Tetrahedron Letters* 7.
6. Karliner, J. and Djerassi, C. (1966) *J. Org. Chem.* **31**, 1945.
7. Budzikiewicz, H., Djerassi, C. and Williams, D. H. (1964) *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. II, p. 122 Holden-Day, San Francisco.
8. Ghulam, A. M. and Hassan, M. G. Al-Hazini (1987) *Phytochemistry* **26**, 225.
9. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
10. Kizu, H., Kitayama, S., Nakatani, F., Tomimori, T. and Namba, T. (1985) *Chem. Pharm. Bull.* **33**, 3324.
11. Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S. (1985) *Phytochemistry* **24**, 2479.
12. Sati, O. P., Rana, U., Chaukiyal, D. C., Madhusudan, K. P. and Bhakuni, D. S. (1987) *Planta Med.* **53**, 530.
13. Kasai, R., Okohara, M., Asakawa, J., Mizutani, K. and Tanaka, O. (1979) *Tetrahedron* **35**, 1427.
14. Tori, K., Seo, S., Shimaka, A. and Tomita, Y. (1974) *Tetrahedron Letters* 4247.
15. Takabe, S., Takeda, T. and Ogihara, Y. (1979) *Carbohydrate Res.* **76**, 101.

Phytochemistry, Vol. 28, No. 2, pp. 647–649, 1989.
Printed in Great Britain.

0031–9422/89 $3.00 + 0.00

E-DIHYDRORHODOPHYTIN, A C_{15} ACETOGENIN FROM THE RED ALGA *LAURENCIA PINNATIFIDA*

MANUEL NORTE, JOSE J. FERNANDEZ, FERNANDO CATALDO and A. G. GONZALEZ

Centro de Productos Naturales Organicos "Antonio Gonzalez" Universidad de La Laguna, Tenerife, Spain

(*Received in revised form* 10 *June* 1988)

Key Word Index—*Laurencia pinnatifida*; Rhodomelaceae; Rhodophyta; marine natural product; acetogenin; dihydrorhodophytin.

Abstract—From the red alga *Laurencia pinnatifida* we have isolated *E* and *Z* dihydrorhodophytin. The structure and absolute configuration of the former have been established by spectroscopical and chemical methods.

INTRODUCTION

Red alga of the genus *Laurencia* are known to produce C-15 acetogenins containing haloethers of different sizes [1]. In continuation of our studies on this type of compound we have reexamined an extract of *L. pinnatifida* collected at Callao Salvaje (Tenerife, Canary Islands). The isolation of *E*- and *Z*-dihydrorhodophytin was accomplished by combined silica gel (2% *n*-hexane–EtOAc), medium pressure and Sephadex LH-20 (*n*-hexane–$CHCl_3$–MeOH, 2:1:1) chromatography. The *Z* isomer **2** was identified by comparison of its physical and spectral properties with those reported [2]. This compound and the previously unpublished *E* isomer were chemically correlated as follows. Catalytic hydrogenation of each isomer over PtO_2 yielded the same compound, decahydrorhodophytin **3**. The ^{1}H and ^{13}C NMR chemical shift assignments of *E*-and *Z*-dihydrorhodophytin, which have not been published, have been obtained using two dimensional NMR experiments, COSY (^{1}H–^{1}H) and COSY (HETCOR) ^{1}H–^{13}C. (Table 1).

It is noteworthy that *E*-and *Z*-dihydrorhodophytin co-occur with *E*-and *Z*-pinnatifidenine in *L. pinnatifida* [3], in view of the biogenesis of the C_{15} halogenated cyclic ethers. The (6*R*, 7*R*)-3*Z*, 9*Z*, 12*Z*-6-acetoxy-7-chloropentadeca-3,9,12-trien-1-yne, isolated from the same alga [3], might be an intermediate in the biosynthetic pathway to a *Z*-pinnatifidenine. For dihydrorhodophytin the intermediate must be the (6*S*, 7*S*) isomer which has not been found previously.