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TRITERPENOIDS FROM *GUETTARDA ANGELICA**

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Key Word Index—*Guettarda angelica*, Rubiaceae, roots, triterpenes, glycoside, 3β-*O*-β-D-glucopyranosyl-quinovic acid, rotundic acid, hederagenin, 3β,23-dihydroxyurs-12-en-28-oic acid

Abstract—From the root bark of *Guettarda angelica* were isolated 3-*O*-β-D-glucopyranosyl-quinovic acid and its aglycone The root wood gave the same glucoside, rotundic acid, hederagenin and 3β,23-dihydroxyurs-12-en-28-oic acid

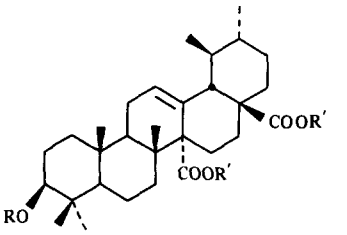
INTRODUCTION

Guettarda angelica Mart is a medicinal plant of the Rubiaceae family which is indigenous to the northeastern and central regions of Brazil The yellow and very bitter root bark of this shrub has been used in folk medicine [1] The aqueous extract of this plant exhibited a hypoglycemic effect on rats [2] No reference could be found describing the chemical constituents of this plant A preliminary chemical screening showed the presence of flavonoids, alkaloids and triterpenoids

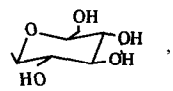
An ethanol extract from the roots of *G angelica* yielded the previously known triterpenoids, quinovic acid (**1**) and its glucoside (**2**) [3–7], and rotundic acid (**3**) [8, 9]. The present communication describes the isolation and structure elucidation of a mixture of methyl hederagenin (**4a**) [10] and its isomer (**5a**) belonging to the ursane group The latter is a new triterpene and it has been characterized as 3β,23-dihydroxyurs-12-en-28-oic acid (**5**)

RESULTS AND DISCUSSION

The ethanol extract of roots of *G angelica* afforded a fraction containing the triterpenes **4** and **5** that were transformed to the methyl esters **4a** and **5a** by methylation with diazomethane Isomeric mixtures of olean-12-enes and urs-12-enes have frequently been isolated from the plant kingdom Separation of these triterpenoids is a challenging problem that remains unresolved However, ^{13}C NMR spectroscopy permits their identification and was used to analyse the components of *Isodon japonicus* Hara tissue cultures [11] Analysis of the ^{13}C NMR spectrum of the fraction obtained from *Guettarda angelica* showed signals due to methyl hederagenin (**4a**) carbons 12 and 13 (Table 1) [12] The remaining signals were assigned to the ursene isomer (**5a**) by considering the differences discernible from the corresponding oleanene carbons, especially the C-18, C-19 and C-20 signals, as well as those of C-12 and C-13 which were of most diagnostic value The signals at δ49 7 and 38 9, assigned to C-5 and C-19 respectively, were in agreement with a triterpenoid structure containing a CH_2OH group at C-4 and a methyl group at C-19 This indicated that the compound was not identical with methyl ursolate [13] or methyl rotundate



1 R = R' = H
1a R = Ac, R' = Me



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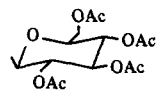
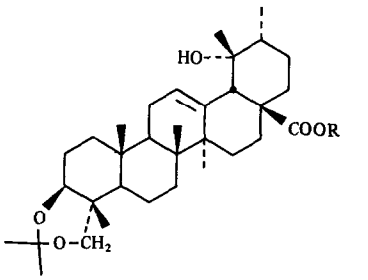
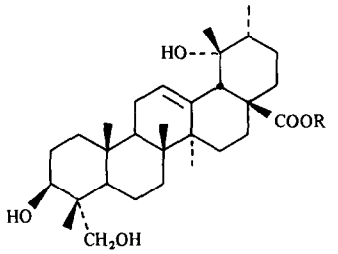


2a R = , R' = Me

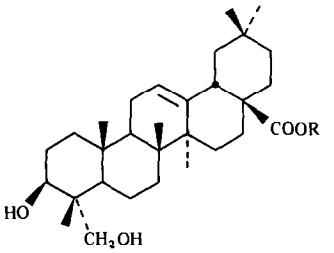


8 R = Me
8a R = H

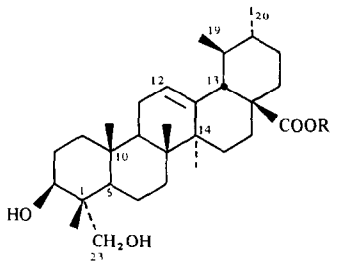
*Based on part of the M Sc thesis submitted by M P S to Universidade Federal do Ceará, Departamento de Química Orgânica e Inorgânica (1981), for prelimary communication see (1982) *Ciência e Cultura* (São Paulo) **34** (Suplemento), 491



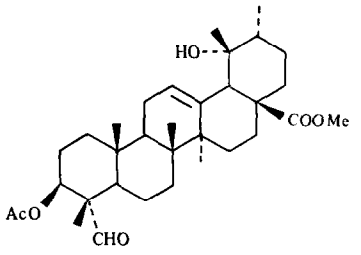
3 R = H
3a R = Me



4 R = H
4a R = Me



5 R = H
5a R = Me



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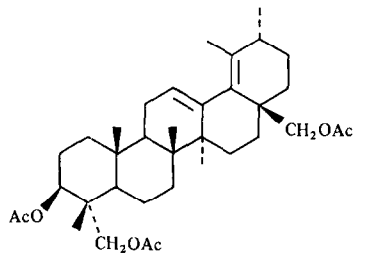

7

[14] Thus, the other component of the fraction of *G angelica* must be 3β,23-dihydroxyurs-12-en-28-oic acid, which is a new natural product The ^{13}C NMR chemical shift values for **5a** are also included in Table 1 Hederagenin is present in many plant species but its ursene isomer (**5a**) has not been reported in the literature

Two new triterpenes, **6** and **7**, were obtained by transformation from methyl rodundate Treatment of methyl rodundate (**3a**) with pyridinium chromate on silica gel [15] and acetylation yielded a new aldehyde (**6**), mp 88–90° In the ^{1}H NMR spectrum of **6** a singlet at δ9 30 was due to an aldehyde proton The absence of a broad

Table 1 ^{13}C NMR spectral data for compounds **4a** and **5a** (25 2 MHz, $CDCl_3$, TMS as internal standard)

Carbon	4a	4a + 5a	5a	Carbon	4a	4a + 5a	5a
1		38 4		16	23 3		24 3
2		26 5		17	46 7		48 2
3		76 4, 77 0		18	41 3		52 8
4		41 7		19	46 0		38 9
5		49 7		20	30 7		38 9
6		18 5		21	33 9		30 7
7		32 5		22	32 7		36 7
8		39 1, 39 3		23		71 4	
9		47 6		24		11 7	
10		36 9		25		15 8, 15 7	
11		23 7, 23 9		26		17 0	
12	122 4		125 5	27	26 0		23 7
13	143 8		138 2	28		178 2, 178 3	
14	41 3		42 0	29	33 2		17 0
15		27 8, 28 2		30	24 0		21 2
				COOMe		51 4	

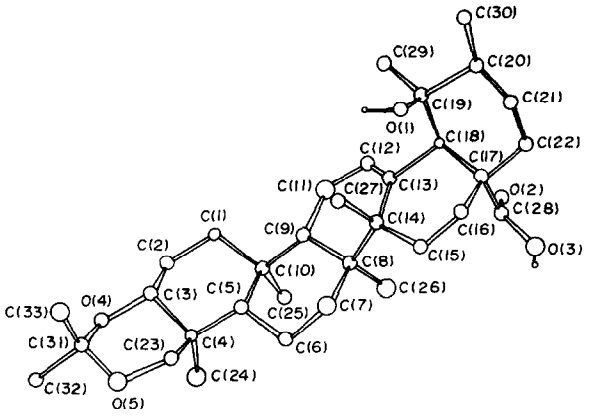

Fig 1 Perspective view of the molecular structure of compound **8a**

singlet at 3 80 due a CH_2OAc group confirmed the structure **6** for the aldehyde

Reduction of methyl rodundate with lithium aluminium hydride followed by acetylation afforded 3β,23,28-tri-*O*-acetyl-urs-12,18-diene (**7**), which occurred with elimination of water during the process The ^{1}H NMR spectrum of **7** showed a vinyl proton at δ5 26 (*m*, H-12) and AB systems at 3 86 and 3 71 (*d*, J = 12 Hz, 23-CH_2OAc), 4 02 and 3.95 (*d*, J = 11 Hz, 28-CH_2OAc) and a methyl group at 1 68 (Me-19) The mass spectrum of **7** showed the characteristic fragmentation of the C-ring of Δ^{12}-pentacyclic triterpenoids giving rise to peaks at *m/z* 308 and 274 [16]

Fractionation of the ethanol extracts from roots of *G angelica* and recrystallization of the product from benzene–acetone yielded the methyl rotundate acetonide (**8**) whose ^{1}H NMR spectrum was identical with that of the acetonide obtained by reacting methyl rotundate with acetone in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulphonic acid [17]

Unequivocal proof of the structure and relative configuration of **8a** was achieved by X-ray crystallographic analysis Three-dimensional X-ray diffraction data was collected using a CAD_4 automatic single crystal diffractometer Unit cell dimensions are a = 11 353 (2), d = 13 111 (4), c = 24 049 (3) Å The space group is $P2_12_12_1$ and Z = 4 The least squares isotropic refinement converged at R = 0 075 with 720 intensities greater than 2σ (I) where σ (I) was estimated counting statistics The structure was solved by direct methods using MULTAN [18] The relative configuration is shown in Fig 1 A more complete and detailed description of the crystal structure will be submitted for publication elsewhere

EXPERIMENTAL

Extraction and isolation of compounds Milled dry root bark (6 5 kg) and root wood (2 8 kg) were separately percolated at room temp with EtOH The EtOH extracts were evaporated to dryness to yield a brown mass (405 g and 95 g, respectively) The root bark extract was washed with 5% aq HCl and the soln obtained was rich in alkaloids This fraction was not analysed The acidic fraction (81 g) obtained after washing of the residue with 5% aq Na_2CO_3 was acetylated as usual and chromatographed over silica gel and then methylated with CH_2N_2 Repeated chromatography on silica gel furnished the acetyl dimethyl ester (**1a**), mp 207–211° (lit [4] 219–222°) and the glucosyl peracetyl dimethyl ester (**2a**), mp 100–105° The root wood extract (95 g) was mixed with silica gel and the powder was eluted with C_6H_{12}, $CHCl_3$, $CHCl_3$–Me_2CO (9 1) and MeOH The $CHCl_3$–Me_2CO fraction (13 g) was chromatographed and the product obtained was methylated with CH_2N_2 Repeated chromatography of the crude product furnished **3a**, mp 244–246° (lit [8] 257°, lit [9] 253–255°) and a mixture of **4a** and **5a**, mp 130–133° ($CHCl_3$–MeOH) ^{1}H NMR (60 MHz, $CDCl_3$) δ5 20 (H-12, *m*), 3 80–3 40 (H-3), 3 53 (OMe, *s*), 3 40 (23-CH_2OH, *s*), 2 88 (H-18, *m*, oleanene group), 2 25 (H-18, *s*, ursene group), 1 07, 1 02, 0 90, 0 82, 0 70 (Me, *s*), MS *m/z* (rel int) 486 (7), 468 (1), 427 (2), 426 (3), 395 (1), 263 (20), 262 (100), 247 (8), 224 (12), 206 (11), 203 (89), 202 (23), 133 (44)

Preparation of aldehyde **6** Compound **3a** (300 mg) in CH_2Cl_2 (300 ml) was treated with pyridinium chromate on silica gel (3 g), prepared by the procedure described elsewhere [15], and AcOH (0 2 ml) The mixture was mechanically shaken for 4 hr at room temp The reaction was monitored by TLC At the end of this period, Et_2O (10 ml) was introduced and, after shaking for another 2 min, filtered Evaporation of the solvent gave a residue (190 mg) that was acetylated (Ac_2O–pyridine) and chromatographed on silica gel Elution with hexane–$CHCl_3$ (1 1) furnished the aldehyde **6**, mp 88–90°

3β,23,28-*Tri-O-acetyl-urs*-12,18(19)-*diene* (**7**) To a soln of **3a** (500 mg) in Et_2O (100 ml) was added $LiAlH_4$ (1 g) dissolved in Et_2O (50 ml) The soln was then shaken for 2 hr at room temp and allowed to stand for 24 hr The reaction mixture was treated with EtOAc and acidified with aq 5% HCl The EtOAc extract was evaporated to dryness and chromatographed on silica gel yielding the acetate (**7**), mp 86–89° ^{1}H NMR (100 MHz, $CDCl_3$) δ5 26 (*m*, H-12), 4 82 (*dd*, J = 5 and J = 10 Hz, H-3) 3 86, 3 71 (AB system, J = 12 Hz, 23-C$\underline{H_2}$OAc), 4 02, 3 95 (AB system, J = 11 Hz, 28-CH_2OAc), 2 07 (*s*, 2Ac), 2 04 (*s*, Ac), 1 68 (*s*, Me-29), 1 06 (*s*, Me-30), 1 04 (*s*, Me-27), 1 00–0 98 (Me-25), 0 86 (Me-24), MS *m/z* (rel int) 582 (17), 540 (3), 523 (5), 522 (9), 509 (25), 308 (5), 274 (7), 232 (7), 247 (18), 248 (7), 215 (33), 201 (100), 188 (13), 43 (99)

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REFERENCES

1 Braga, R (1976) *Plantas do Nordeste Especialmente do Ceará* Mossoró, Escola de Agricultura, Mossoró V 42, p 39
2 Tavares, Z M and Sousa, M P (1976) *Ceará Médico* **1**, 26
3 Tschesche, R, Duphorn, I and Snatzke, G (1963) *Ann Chem* **167**, 151
4 Hui, W H and Yee, C W (1968) *Aust J Chem* **21**, 543
5 Banerji, N and Dutta, N L (1976) *Indian J Chem* **14B**, 614
6 Raffouf, R F, Le Quesne, P W and Ghash, P C (1978) *J Nat Prod (Lloydia)* **41**, 432
7 Banerji, N (1978) *J Indian Chem Soc* **55**, 275
8 Oyama, T, Aoyama, H, Yamada, K, Nitsuhashi, T and Sugiyama, N (1968) *Tetrahedron Letters* 4639
9 Takani, M, Kubota, K, Nozawa, M, Ushun, T and Takakashi, K (1977) *Chem Pharm Bull* **25**, 981
10 Gaudemer, A, Polonsky, M^{me} J and Wenkert, E (1964) *Bull Soc Chim Fr* 407
11 Seo, S, Tomita, Y and Tori, K (1975) *Tetrahedron Letters* 7
12 Tori, K, Seo, S, Shimoaka, A and Tomita, Y (1974) *Tetrahedron Letters* 4227
13 Wehrli, F W and Nishida, T (1979) in *Progress in the*

Chemistry of Organic Natural Products (Zechmeister, L., ed.) Vol 36, p 99 Springer, Wien

14 Takahashi, K and Takani, M (1978) *Chem Pharm Bull* **26**, 2689

15 Singh, R P, Subbarao, H N and Dev, S (1979) *Tetrahedron* **35**, 1789

16 Budzikiewicz, H, Wilson, J M and Djerassi, C (1963) *J Am Chem Soc* **85**, 3688

17 Tsuda, Y and Fujimoto, T (1970) *J Chem Soc Chem Commun* 260

18 Germain, G, Main, P and Woolfson, M M (1971) *Acta Crystallogr* **A27**, 368