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^{13}C NMR ANALYSIS OF MONODESMOSIDIC SAPONINS FROM *GOMPHRENA MACROCEPHALA*

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Key Word Index—*Gomphrena macrocephala*; Amaranthaceae; tuberous roots, monodesmosidic saponins with a homoannular diene or γ-lactone systems.

Abstract—The known ^{13}C NMR spectral data of triterpenoid sapogenins and saponins was conveniently applied for direct structure identification of mixtures containing 28-*O*-β-D-glucopyranosyl-ester-oleanolic acid and 28-*O*-β-D-glucopyranosyl-ester-olean-9(11),12-diene, 3-*O*-β-D-glucopyranosyl-erythrodiol and 3-*O*-β-D-glucopyranosyl-11,12-epoxy-olean-28,13-olide, the latter compound and 3-*O*-β-D-glucopyranosyl-olean-28,13-olide from *Gomphrena macrocephala*.

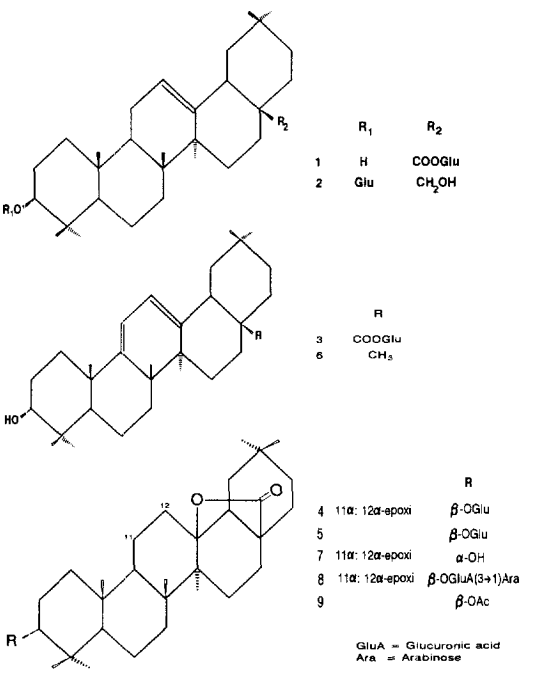
INTRODUCTION

Gomphrena macrocephala St.-Hill. is a perennial herb that grows spontaneously in Brazilian cerrado, a floristically and physiognomically diverse savanna vegetation [1]. Popularly known as 'paratudo', its tuberous roots are widely used in folk medicine, mainly as a tonic and stimulant [2]. In an earlier communication, the isolation of ecdysterone and chikusetsusaponin IVa from tuberous roots of this plant has been reported [3]. A significant molluscicidal activity against *Biomphalaria glabrata* was detected in the ethanolic and aqueous crude extracts [4]. We now report the use of ^{13}C NMR spectroscopy to identify triterpenoid glucoside mixtures from ethyl acetate extract of *G. macrocephala* that also displayed molluscicidal activity.

RESULTS AND DISCUSSION

The 80% ethanol extract of the roots of *G. macrocephala* was extracted successively with ethyl acetate and butanol. The ethyl acetate and butanol fractions were bioassayed against *Biomphalaria glabrata* snails and both showed molluscicidal activity, exhibiting LD_{90} values of 19.25 and 21.12 ppm, respectively.

The ethyl acetate fraction on chromatographic purification led to the isolation of the 28-*O*-β-D-glucopyranosyl-ester-oleanolic acid (**1**), 3-*O*-β-D-glucopyranosyl-erythrodiol (**2**) and three triterpenoid glucosides (**3**, **4** and **5**) that seem to be new compounds according to the ^{13}C NMR chemical shifts [5]. The saponins **1** and **2** have already been isolated from *Panax japonicum* and *Androsace septentrionalis* [6], respectively. The fraction 6 obtained as described in the Experimental section appeared pure on TLC.



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However, analysis by ^{13}C NMR spectroscopy using DEPT techniques (Table 1) showed this fraction contained in fact a mixture of compound **1** and an unknown substance. These spectral data indicated the occurrence of additional chemical shifts at δ 154.7 (C), 115.4 (CH), 120.5 (CH) and 144.5 (C), suggesting the presence of two double bonds and δ 19.8 (CH_3), 20.0 (CH_3), corresponding to two methyl groups. Thus, the signals in the spectra suggested that the genin was comparable to the compound **6** [5]. Nevertheless, two anomeric signals almost superimposable at δ 94.0 and 93.9 were observed, indicating that a β-glucopyranosyl substituent must be attached to the carboxyl group at C-28. These data indicated that fraction 6 contains **1** and 28-*O*-β-D-glucopyranosyl-ester-olean-9(11),12-diene (**3**).

Fraction 19 showed one spot on TLC but ^{13}C NMR chemical shifts indicated the presence of two compounds one of them being identical to 3-*O*-β-D-glucopyranosyl-erythrodiol (**2**) (Table 2). The other compound showed an anomeric carbon signal at δ 106.7 and five additional signals corresponding to the glucopyranosyl moiety. The aglycone moiety showed a quaternary carbon signal at δ 179.1 which could be attributed to a carboxyl ester or γ-lactone. The occurrence of signals at δ 87.7 (C), 57.4 (CH) and 52.9 (CH) could correspond to the presence of an epoxy group in C-11 and C-12, similar to the chemical shifts of **7** and **8**. No olefinic carbon signal was observed. Thus, this mixture was presumed to contain **2** and 3-*O*-β-D-glucopyranosyl-11,12-epoxy-olean-28,13-olide (**4**) which has not been reported previously.

Table 1. ^{13}C NMR chemical shifts of a mixture of **1** and **3** in $CDCl_3+CD_3OD$, **6** in $CDCl_3$

Carbon	DEPT	**1** + **3**		**6** [5]
1	CH_2	38.4	—	38.8
2	CH_2	27.3	27.4	27.9
3	CH	78.6	78.2	78.6
4	C	38.3	39.2	38.9
5	CH	55.0	49.2	51.2
6	CH_2	18.0	18.1	18.4
7	CH_2	31.7	32.1	32.2
8	C	38.4	36.8	37.0
9		47.4CH	154.7C	154.3C
10	C	36.7	41.6	40.7
11		22.7CH_2	115.4CH	115.8CH
12	CH	122.4	120.5	120.8
13	C	143.1	144.5	147.1
14	C	41.0	41.6	42.8
15	CH_2	27.7	26.7	25.7
16	CH_2	22.7	26.7	27.3
17	C	45.6	46.7	32.2
18	CH	41.5	41.1	45.6
19	CH_2	46.7	45.7	46.9
20	C	30.3	30.4	31.1
21	CH_2	33.6	33.7*	34.7
22	CH_2	32.7	33.6*	37.2
23	CH_3	29.4	27.8	28.8
24	CH_3	15.2	15.1	15.1
25	CH_3	15.0	19.8	20.1
26	CH_3	16.5	20.0	21.0
27	CH_3	26.5	25.6	25.3
28		176.9C	176.6C	28.3CH_3
29	CH_3	32.5	32.8	23.7
30	CH_3	23.1	23.3	33.2
28-*O*-Glu				
1′	CH	94.0	93.9	—
2′	CH	72.2	72.3	—
3′	CH	76.5	76.4†	—
4′	CH	69.6	69.7	—
5′	CH	76.5	76.5†	—
6′	CH_2	61.3	61.5	—

*†Assignments may be reversed within a column.
Glu = Glucose.

Table 2. ^{13}C NMR chemical shifts of compounds **2**, **4**, **5**, **7**, **8** in C_5D_5N and **9** in $CDCl_3$

Carbon	DEPT	**2**	**4**	**5**	**7** [5]	**8** [9]‡	**9** [13]
1	CH_2	38.5	39.8	39.1	33.0		38.7
2	CH_2	26.3	27.0	27.8	27.5		23.9
3	CH	88.8	89.1	89.0	75.1	89.1	80.8
4	C	39.3	39.5	39.6	37.0	39.5	37.9
5	CH	55.6	55.9	56.2	48.6		55.2
6	CH_2	18.4	18.5	17.7	17.3		17.7
7	CH_2	33.1	31.5	34.5	31.5		34.2
8	C	39.6	41.0*	42.2	40.9	41.0*	42.3
9	CH	47.8	49.8	51.1	49.8	49.9	50.7
10	C	36.8	36.7	36.7	37.0	36.5	36.8
11		$23.5CH_2$	52.9†CH	$19.0CH_2$	52.9CH	52.6†	$19.7CH_2$
12		122.4CH	57.4†CH	$28.0CH_2$	57.4CH	57.3†	$27.4CH_2$
13	C	144.8	87.7	91.7	88.0	87.5	91.8
14	C	42.0	41.6*	42.5	41.9	41.7*	42.3
15	CH_2	28.3	26.3	26.3	27.0		26.5
16	CH_2	26.4	21.7	20.4	21.7		20.9
17	C	46.4	44.1	44.2	44.1	44.1	44.1
18	CH	41.8	51.1	50.1	51.1	51.2	50.4
19	CH_2	46.4	37.0	38.1	38.0		37.5
20	C	30.8	31.0	31.7	31.6	31.5	31.5
21	CH_2	34.1	34.4	32.8	34.4		33.5
22	CH_2	29.8	27.6	30.7	27.7		31.4
23	CH_3	28.1	28.3	28.3	29.1		28.0
24	CH_3	16.9	16.5	16.5	22.2		16.5
25	CH_3	15.3	17.5	16.5	17.3		16.1
26	CH_3	17.3	18.9	17.3	18.8		18.3*
27	CH_3	26.1	20.2	17.3	20.5		18.4*
28		$73.5CH_2$	179.1C	179.1C	180.0C	178.5C	180.3C
29	CH_3	33.2	33.4	23.9	33.6		23.7
30	CH_3	23.7	23.5	24.1	23.5		23.7
3-*O*-Glu							
1′	CH	106.6	106.7	106.7	—		—
2′	CH	75.1	75.2	75.2	—		—
3′	CH	76.5	77.4	77.4	—		—
4′	CH	73.6	73.7	73.7	—		—
5′	CH	77.9	78.1	78.1	—		—
6′	CH_2	61.5	61.6	61.6	—		—

*† Assignments may be reversed within a column.
‡ Main chemical shifts of the aglycone moiety.

The ^{13}C NMR spectrum of fraction 20 showed chemical shifts similar to those of **4**. However, a singlet was observed at δ 91.7 which could be attributed to a tertiary carbinyl carbon. These data were observed in γ-lactonized triterpene such as **9**. Thus, this mixture might correspond to the 3-*O*-β-D-glucopyranosyl-olean-28,13-olide (**5**) and 3-*O*-β-D-glucopyranosyl-11,12-epoxy-olean-28,13-olide (**4**).

Triterpenoid glycosides with a homoannular diene [C-9(11),12] or γ-lactone systems at C-13,28 have been isolated from *Corchorus acutangulus* [7] and *Momordica cochinchinensis* [8], respectively. On the other hand, compounds possessing a diene moiety as above have been reported as derivatives of saikosaponins under pseudo-physiological and chemical conditions [9]. Furthermore, triterpenes with γ-lactone moieties of the ursane and oleanane series have been formed by biogenetic type photochemical conversion [10] and chemical reactions [11, 12]. These observations suggest that the compounds **3**, **4** and **5** could possibly be artefact products obtained during the separation process in which ether was used. However, an additional study must be undertaken in order to clarify this point.

EXPERIMENTAL

General. Mps: Uncorr, ^1H NMR (200 MHz) and ^{13}C NMR (50 MHz): $CDCl_3+CD_3OD$ and C_5D_5N and chemical shifts expressed with ref. to deuterated solvents. Molluscicidal testing was achieved with *Biomphalaria glabrata* snails according to ref. [4].

Plant material. Tuberous roots of *G. macrocephala* St.-Hill. were collected from preserved areas of Brazilian cerrado kept at the Estação Ecológica e Experimental de Mogi-Guaçú, São Paulo State, Brazil. A voucher specimen (SP 167468) has been deposited in the Herbarium Maria Eneida P. K. Fidalgo of Instituto de Botânica de São Paulo, Brazil.

Extraction and isolation of saponins. Sliced tuberous roots (6 kg) were macerated with 80% EtOH. The resulting extract was partially concd and successively partitioned with EtOAc and *n*-BuOH (satd with H_2O). Evapn yielded EtOAc (130 g) and BuOH (222 g) extracts. Part of the former fr. (1.7 g) was sepd by silica gel CC with EtOAc–MeOH (1:0 → 17:3) followed by EtOAc–MeOH–H_2O (77:15:1 → 77:15:8) and finally with $CHCl_3$–MeOH–H_2O (16:9:2) given frs 1–16. Fr. 4 (204 mg) was purified by silica gel CC with EtOAc–MeOH–H_2O (90:10:2.5) to yield 40 mg of pure **1**. Silica gel CC ($CHCl_3$–MeOH–H_2O 16:9:2) of fr. 11 (220 mg) followed by purification of the main fr. (80 mg) on silica gel CC with EtOAc–MeOH–H_2O (77:15:8 + 0.5% HOAc) yielded **2** (25 mg). Another part of the EtOAc extract (5 g) was treated with $CHCl_3$ yielding an insoluble residue (3.2 g). This was subjected to CC on DIAION-HP-20 eluted with mixts of H_2O and MeOH in decreasing polarity to give 6 frs. Fr. 5 (946 mg) obtained in H_2O–MeOH (1:4) was subjected to CC on silica gel in Et_2O with gradient of $CHCl_3$–MeOH–H_2O-*iso*-PrOH (8:8:8:1, lower phase) followed by the later solvent mixture (9:12:8:2, lower phase) and 23 frs were collected. Fr. 6 was further purified by CC [silica gel 60, EtOAc–MeOH–H_2O (18:2:1)] yielding a mixt. of **1** and **3** (23 mg). Fr. 19 yielded, after CC [silica gel 60, EtOAc–MeOH–H_2O (77:15:8) containing 0.5% HOAc] a mixt. of **2** and **4** (45.7 mg). Fr. 20, after TLC [silica gel 60, $CHCl_3$–MeOH–H_2O (16:9:2)] looked to be composed of only one product. However, ^{13}C NMR examination of this compound indicated an inseparable mixt. of **4** and **5** (71 mg).

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