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A PREGNANE TRIGLYCOSIDE ESTER FROM *DREGEA SINENSIS* VAR *CORRUGATA*

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Key Word Index—*Dregea sinensis* var *corrugata*, Asclepiadaceae, dregeoside, steroid, pregnane glycoside ester

Abstract—A new pregnane glycoside ester, dregeoside, was isolated from the dried rhizome of *Dregea sinensis* var *corrugata* On the basis of chemical reactions and spectroscopic evidence, the structure was established as 12-*O*-benzoyl-drevogenin-3-*O*-*β*-D-oleandropyranosyl(1→4)-*O*-*β*-D-cymaropyranosyl(1→4)-*O*-*β*-D-cymaropyranoside

INTRODUCTION

In a previous paper [1], we reported the isolation and structure elucidation of a drevogenin from the rhizomes of *Dregea sinensis*. As a continuation of the studies on this plant, we present the spectral and chemical evidence for the structure of a new triglycoside (**1**)

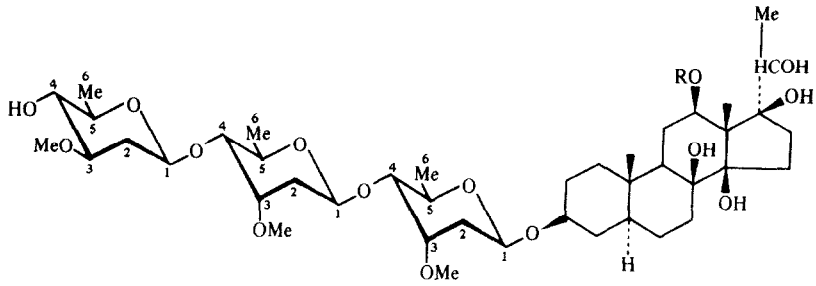
RESULTS AND DISCUSSION

Dregeoside (**1**) was isolated by gel column chromatography and reverse-phase chromatography. The mass spectrum of compound **1** indicated that molecular formula was $C_{49}H_{76}O_{16}$ (FAB-MS $[M]^+$ at *m/z* 920). The UV spectrum showed absorption at 230 nm Its IR spectrum showed the presence of methoxy group (2925, 1440 cm^{-1}) and –C–O–C–O– (1195, 1160, 1080, 1050 cm^{-1}) Confirmation of the triglycoside structure of compound **1** and the position of its monobenzoate ester group on the genin moiety was provided by the ^1H NMR (400 MHz) spectrum of **1** which indicated the presence of three methoxy group at 3.38 (3H, *s*, OMe), 3.45 (6H, *s*, 2 × OMe) and three methyl group at 1 23 (6H, *d*, $J=6$ Hz, scc. 2 × Me), 1.34 (3H, *d*, $J=6$ Hz, sec. Me) A methyl group and a methoxy group could be assigned to C-3 and C-6, respectively, of a deoxy sugar

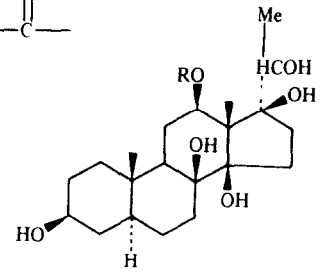
As cymaropyranose possesses only two hydroxy groups at C-1 and C-4, the sugar sequence in **1** was linear We assigned the ^{13}C NMR signals of the sugar chain in **1** as shown in Table 1 in comparison with the data on the ^{13}C NMR chemical shifts of methyl *β*-D-cymaroside and *α*,*β*-D-oleandroside [2] [3] From the ^{13}C NMR chemical shifts of the anomeric carbons the D-cymarose and D-oleandrose moieties in **1** are suggested to have a *β*-configuration at C-1. The ^{13}C NMR data of **1** is presented in Table 1. Mass spectral fragment ion peaks at *m/z* 145 ($C_7H_{13}O_3$), 144 ($C_7H_{12}O_3$), and 499 ($C_7H_{13}O_3$–$C_7H_{12}O_3$–$C_7H_{12}O_4$) suggested that there were three deoxy sugars in the molecule.

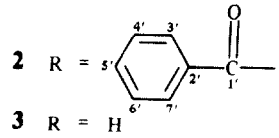
Mild acid hydrolysis of the acetate of **1** with dilute sulphuric acid yielded 4-*O*-acetyl-oleandrose, cymarose and the monoacetate of **2** which was identical with an authentic sample as determined by TLC and GLC Oleandrose was indicated to be the terminal sugar

Mild acid hydrolysis of **1** using the earlier reported method of Mannich and Siewert [4] yielded drevogenin (**2**) and cymarose (**4**), oleandrose **5** ($[\alpha]_D$ mp, TLC) [5]. Alkaline hydrolysis of **2** yielded deacyldrevogenin (**3**), which was identical with authentic dihydrosarcostin (mp, mmp, and IR) [6]. The monobenzoate nature of the ester function in **1** was supported by its IR, UV (λ_{max}^{EtOH} 282 nm, log *ε* 3.05) and ^1H and ^{13}C NMR spectral data. The difference of $C_{21}H_{36}O_9$ between the formula of glycoside **1** and its aglycone **2** indicated that **1** was a triglycoside The same conclusion could be drawn from the mass spectrum of **1** which recorded fragment ions for a trisaccharide unit (*m/z* 450) and the genin moiety (*m/z* 470) besides the prominent fragment ions of drevogenin monobenzoate giving ions for benzoic acid at *m/z* 122 and the other expected ions of the drevogenin moiety including the fragment ions due to the sequential losses of its four molecules of water at *m/z* 452, 434, 416, and 398. In



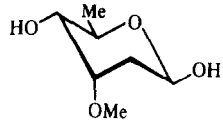
1 R =





2 R =

3 R = H



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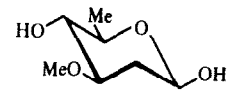

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the light of the above evidence the structure of dregeoside was established as 12-*O*-benzoyl-drevogenin-3-*O*-*β*-D-oleandropyranosyl(1→4)-*O*-*β*-D-cymaropyranosyl (1→4)-*O*-*β*-D-cymaropyranoside

EXPERIMENTAL

Mps uncorr, ^{1}H and ^{13}C NMR 400 MHz, TMS as int standard TLC and CC used $CHCl_3$–MeOH (49 1), reverse phase-CC MeOH–H_2O (4 1)

Plant extraction Shade-dried powdered rhizome (5 2 kg) of *Dregea sinensis* var *corrugata* were extrated with EtOAc on a hot bath The aq concentrate was fractionated with different organic solvents to afford a petrol extract (20 g) and a $CHCl_3$ extract (11 5 g), i e crude glycoside, which was subjected to CC on silica gel with $CHCl_3$–MeOH (49 1) and further on reversed-phase gel with MeOH–H_2O (4 1) to afford **1** (120 5 mg, yield 0 0011%)

Dregeoside **1**, Mp 125–128°, $[\alpha]_D^{25}$ +43 2° (MeOH, *c* 0 15) (Found C, 63 91, H, 8 26, $C_{49}H_{76}O_{16}$ requires C, 63 81, H, 8 15%) It showed positive Liebermann–Burchard and Keller–Kiliani reactions UV λ_{max} nm (log ε) 231 (4 23), 274 (3 12), 282 (3 05) IR ν_{max} cm^{-1} 3540 (ass OH group), 1710 (C=O of benzoate ester), 1368 (Me def), 710 (C-H def arom) ^{1}H NMR (400 MHz, $CDCl_3$) 7 49 (2H, *t*, *J* = 7 9 Hz, 4′, 6′-CH), 7 62 (1H, *tt*, *J* = 7 9, 1 2 Hz, 5′-CH), and 8 05 (2H, *dd*, *J* = 7 9, 1 2 Hz, 3′, 7′-CH), 4 47 (1H, *dd*, *J* = 10, 2 Hz, 1-CH of *β*-D-cymaropyranose), 4 74 (1H, *dd*, *J* = 10, 2 Hz, 1-CH of *β*-D-cymaropyranose), and 4 82 (1H, *dd*, *J* = 10 2 Hz, 1-CH, of *β*-D-oleandropyranose), 4 70 (1H *dd*, *J* = 9, 5 Hz, 12-H), 4 80 (1H, *q*, *J* = 6 5 Hz, 20-H), 3 39 (3H, *s*, OMe), 3 44 (3H, *s*, 2 × OMe), 1 34 (3H, *d*, *J* = 6 Hz, sec Me), 1 25 (6H, *d*, *J* = 6 Hz, sec 2 × Me) 1 24 (3H, *s*, 18-Me), 0 96 (3H, *s*, 19-Me), FABMS *m/z* 920 $[M]^+$, 488 $[M-\text{trisaccharide}]^+$ (3), 470 $[488-H_2O]^+$ (6), 452 $[488-2H_2O]^+$ (2), 443 $[488-45]^+$ (4), 383 $[488-105]^+$ (8), 366 $[488-\text{BzOH}]^+$ (100), 348 $[488-\text{BzOH}-H_2O]^+$ (5) 321 $[488-\text{BzOH}-\text{MeCHOH}]^+$ (4), sugar fragments 450 (dicymarose and oleandrose)$^+$ (3), 354 $[450-3\text{MeOH}]^+$ (24), 290 (rearranged disaccharide fragment]$^+$ (100), 162 [cymarose or oleandrose]$^+$ (21), 130 $[162-\text{MeOH}]^+$ (22), 86 (21)

Mild acid hydrolysis of **1**. A soln of **1** (20 mg) in MeOH (10 ml) was mixed with 0 05 M H_2SO_4 (1 5 ml) and warmed for 30 min at 50°, MeOH was then removed under red pres The aq concentrate was repeatedly extracted with $CHCl_3$ and the organic layer washed in turn with H_2O, 1 M Na_2CO_3 and H_2O, dried over Na_2SO_4 and evapd to afford the genin **2** (12 mg) $[\alpha]_D^{25}$ +63 (MeOH, *c* 0 2) ^{1}H NMR (400 Hz) of **2** 8 05–7 49 (5H, *m*, aromatic), 1 91 (3H, *s*, 21-Me), 0 96 (3H, *s*, 19-Me), 1 24 (3H, *s*, 18-Me), 4 80 (1H, *q*, *J* = 6 5 Hz, 20-H) The aq. hydrolysate was neutralized with freshly pptd $BaCO_3$, filtered and concd under red pres to yield the syrupy soln This gave a positive Keller-Kiliani reaction, the sugars were identical with authentic samples by comparison on TLC and GLC of the TMS-sugar

Table 1 ^{13}C NMR chemical shifts of dregeoside ($CDCl_3$, TMS, ppm)

Aglycone moiety			Sugar moiety		Methyl glycoside
C-1	38 12 *t*		Cymarose		V
2	33 13 *t*	C-1	96 65	C-1	99 4
3	70 91 *d*	2	37 30	2	35 1
4	38 92 *t*	3	77 40	3	78 5
5	45 41 *d*	4	83 70	4	74 0
6	34 01 *t*	5	68 75	5	71 0
7	34 23 *t*	6	18 58	6	18 9
8	76 81 s	C-3-OMe	58 47	C-1-OMe	57 8
9	46 62 *d*		Cymarose	C-1-OMe	56 0
10	38 13 *s*	C-1	100 30		VII
11	28 81 *t*	2	38 01	C-1	101 0
12	74 25 *d*	3	77 40	2	36.6
13	56.05 *s*	4	83 14	3	81 3
14	83.80 *s*	5	68 75	4	76 2
15	34 05 *t*	6	18 58	5	72 6
16	32 75 *t*	C-3-OMe	58 47	6	18 4
17	88 15 s		Oleandrose	C-1-OMe	56 9
18	11 82 *q*	C-1	102.04	C-3-OMe	56 0
19	16 15 *q*	2	37 30		IX
20	69 52 *d*	3	81 20	C-1	98 7
21	16 70 *q*	4	76 77	2	35 1
C-1′	165 85 *s*	5	73 50	3	79 0
2′	130 32 *s*	6	18 09	4	76 6
3′	130 32 *d*	C-3-OMe	56 80	5	68 4
4′	129 21 *d*			6	18 4
5′	133 42 *d*			C-1-OMe	56 9
6′	129.21 *d*			C-3-OMe	54 3
7′	130 32 *d*				

cymarose (R_f 0 46) and oleandrose (R_f 0 28); relative retention time (min): 0 44 (cymarose), 0 37 (oleandrose)

Alkaline hydrolysis of compound **2**. Compound **2** (8 mg) was dissolved in 5% methanolic KOH (1 5 ml) and refluxed for 5 hr After adding H_2O (1 0 ml) MeOH was removed under red pres The aq concentrate was extracted with $CHCl_3$–MeOH (9 1), dried over Na_2SO_4, filtered and evapd to dryness yielding **3** (4 0 mg) which crystallized from MeOH, mp 245–248°, $[\alpha]_D^{25}$ +51° (MeOH, *c* 0 15). It was identified as deacyldrevogenin by comparison with an authentic sample of dihydrosarcostin ($[\alpha]_D$, TLC, mmp) [6]

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