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STEROIDAL ALKALOIDS OF *MARSDENIA ROSTRATA*

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Key Word Index—*Marsdenia*; Asclepiadaceae; steroids; steroidal alkaloids; rostratine; dihydrorostratine; anabasine; metaplexigenin.

Abstract—Some of the main constituents of *Marsdenia rostrata*, originating from two localities, were isolated and identified. The sample from the Toonumbar State Forest in northern New South Wales contains the known alkaloid anabasine and two new steroidal ester alkaloids, rostratine and dihydrorostratine. They were identified as *O*-acetyl-*O*-nicotinoylsarcostin and *O*-acetyl-*O*-nicotinoyldihydrosarcostin. The sample from the South Coast of N.S.W. yielded the two new ester alkaloids and a number of neutral polyhydroxy pregnane aglycones, one of which has been identified as metaplexigenin, but there was no anabasine.

INTRODUCTION

Marsdenia rostrata R.Br., a robust twiner of the Asclepiadaceae, is widely distributed along the coast and coastal mountains of Eastern Australia. Its constituents are of interest because of its reported toxicity to grazing stock.[1,2] Members of the genus *Marsdenia* which have been studied previously, viz. *M. condurango* Reich.,[3,4] *M. tomentosa* Decne.[5,6] and *M. erecta* R.Br.,[7] yielded glycosides or ester aglycones derived from various polyhydroxy pregnanes. However, there appears to be no record of alkaloid isolation from any *Marsdenia* species although field tests reported the presence of alkaloids in several species including *M. rostrata*.[2,8,9]

RESULTS AND DISCUSSION

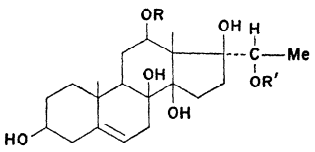
Extraction of *M. rostrata* from the Toonumbar State Forest, N.S.W., yielded 0·45% of basic material, while the Mt. Keira sample, Wollongong, N.S.W., contained 0·29% basic material and 2·45% neutral aglycones.

Column chromatography of the bases obtained from the Toonumbar State Forest collection gave two main fractions. The major component of the first fraction was identified as racemic anabasine (3-(2′-piperidyl)-pyridine), an alkaloid which is known to racemise easily.[10] Anabasine was absent from the plant material collected in the Wollongong area.

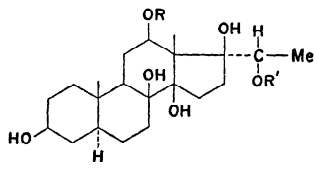
* From the Ph.D. thesis of R.E.S. (1972).

[1] E. HURST, *The Poison Plants of New South Wales*, Poison Plants Committee N.S.W., Sydney (1942).
[2] L. J. WEBB, *Guide to the Medicinal and Poisonous Plants of Queensland*. C.S.I.R. Bulletin No. 232, Commonwealth of Australia, Melbourne (1948).
[3] K. HAYASHI and H. MITSUHASHI, *Chem. Pharm. Bull. Tokyo* **16**, 2522 (1968).
[4] R. TSCHESCHE, P. WELZEL and G. SNATZKE, *Tetrahedron*, **21**, 1777 (1965).
[5] H. MITSUHASHI, T. SATO, T. NOMURA and I. TAKEMORI, *Chem. Pharm. Bull. Tokyo* **13**, 267 (1965).
[6] Y. SHIMIZU, Y. SATO and H. MITSUHASHI, *Chem. Pharm. Bull. Tokyo* **15**, 2394 (1967).
[7] A. SANER, C. ZERLENTIS, W. STOCKLIN and T. REICHSTEIN, *Helv. Chim. Acta* **53**, 221 (1970).
[8] L. J. WEBB, *Australian Phytochemical Survey, Part I*. C.S.I.R.O. Aust. Bulletin No. 241 (1949).
[9] L. J. WEBB, *Australian Phytochemical Survey, Part II*. C.S.I.R.O. Aust. Bulletin No. 268 (1952).
[10] L. MARION, *The Alkaloids* (edited by R. H. F. MANSKE and H. L. HOLMES), Vol. 1, p. 248, Academic Press, New York (1950).

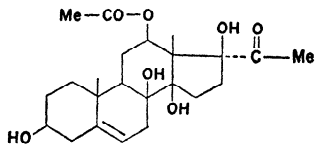
Rostratine (Ia) and dihydrorostratine (IIa) were isolated both from the second fraction of the Toonumbar State Forest collection and from the basic aglycone mixture of the Mt. Keira collection. TLC examination showed one main spot which gave only a faint coloration with iodine vapour but an intense blue colour in the Liebermann–Burchard test.[11] Crystallization of this material from acetone to constant m.p. gave colourless prisms m.p. 264°, $[\alpha]_D^{25}$ +18°. The UV demonstrated the presence of a pyridine moiety in the molecule and the IR spectrum showed hydroxyl absorption at 3460 and 3520 cm^{-1} together with ester carbonyl peaks at 1730 and 1715 cm^{-1} and an aromatic double bond peak at 1600 cm^{-1}.



(Ia) R,R' = Acetyl, nicotinoyl
(Ib) R = R' = H
(Ic) R,R' = Acetyl, benzoyl



(IIa) R,R' = Acetyl, nicotinoyl
(IIb) R = R' = H



(III)

Although the compound appeared homogeneous by TLC the MS and elemental analysis suggested that the compound was a mixture of two components $C_{29}H_{39}NO_8$ and $C_{29}H_{41}NO_8$. The MS showed weak signals both at *m/e* 529 and 531 in the ratio of about 2:1. These peaks were too small for accurate MS measurements but their composition could be inferred from the prominent M-60 (acetic acid) signals at *m/e* 469·2465 ($C_{21}H_{35}NO_6$) and *m/e* 471·2658 ($C_{27}H_{37}NO_6$). The remainder of the spectrum was extremely complex but the metastable mode results shown in Table 2 indicated numerous losses of Me and H_2O, characteristic of polyhydroxypregnane derivatives while prominent neutral fragments of masses 60 and 123 indicated acetyl and nicotinoyl ester substituents.

TABLE 1. NMR SPECTRA OF SARCOSTIN AND DERIVATIVES

Compound	δ-Values relative to tetramethylsilane							
	3α-H	12α-H	20-H	6-H	19-Me	18-Me	21-Me	Acetyl group
Sarcostin[12] (Ib)	3·9*	3·98*	4·44 *q*	5·47 *m*	1·47 *s*	1·94 *s*	1·52 *s*	—
5α-Dihydrosarcostin[12] (IIb)	3·8*	3·88 *q*	4·40 *q*	—	1·26 *s*	1·90 *s*	1·47 *d*; 6·5 Hz	—
O-Acetyl-*O*-benzoylsarcostin[12] (Ic)	3·5 *m*	4·9*	4·6*	5·32 *m*	1·14 *s*	1·68 *s*	1·15 *d*; 6·0 Hz	1·90 *s*
O-Acetyl-*O*-nicotinoyl-(sarcostin-5α-dihydrosarcostin) mixture	3·55 *m*	4·90 *q*	4·76 *q**	5·38	1·16 *s*	1·56 *s*	1·42 *d*; 6·0 Hz	1·96 *s*

* Indicates position of signal not clear because of overlapping; *s*—singlet; *d*—doublet; *q*—quartet; *m*—multiplet.

Alkaline hydrolysis of the genin gave nicotinic acid identified by comparison with an authentic specimen and a two component mixture which was shown by its MS to be a mixture of sarcostin (Ib) and 5α-dihydrosarcostin (IIb) in the ratio of about 2:1. Catalytic hydrogenation of the mixture gave 5α-dihydrosarcostin.

[11] C. MIHALEC, V. JIRGL and J. PODZIMEK, *Experientia* **13**, 242 (1957).

The NMR spectrum of the alkaloid mixture showed four aromatic proton signals in the characteristic pattern of the 3-substituted pyridine system and a 3 proton acetyl methyl signal at δ 1·96. There were further 3 proton signals at δ 1·16 (*s*), δ 1·42 (*d*, *J* 6·0 Hz) and δ 1·56 (*s*) which can be assigned to the C19, C21 and C18 methyl groups of the sarcostin ring system, a very broad one proton signal at δ 3·55 which can be assigned to the C3-α proton and a signal at δ 5·38 which only integrated to about 2/3 proton (based on total integral of the aromatic protons) due to the C6 vinylic proton of the sarcostin portion of the mixture. Two overlapping quartet signals centred at about δ 4·9 and 4·76, corresponding to C12-H and C20-H can be used to assign the ester functions to the hydroxyls at C12 and C20. In sarcostin[12] these signals occur as quartets at δ 3·98 and 4·44; on esterification they are shifted downfield to between δ 4·5 and 5·0. These assignments correspond (Table 1) with those reported for the closely analogous structure of *O*-acetyl-*O*-benzoylsarcostin (Ic) isolated by Reichstein *et al.* from *Sarcostemma viminale*[12] and *Asclepias lilacina.*[13,14] It has not been possible to determine the relative positions of the ester substituents.

TABLE 2. MS METASTABLE MODE RESULTS ROSTRATINE
(peak resulting from parent at *m/e* 529)

Daughter	Measured parent	Assigned parent	Neutral fragment Mass	Comp.
469	530·0	529	60	$C_2H_4O_2$
454	469·4	469	15	Me
	515·3	514	60	
436	453·4	454	18	H_2O
	496·6	496	60	
	469·6	469	33	
	513·8	514	78	
418	434·2	433	15	
	452·3	451	33	
	479·0	478	60	
	495·3	496	78	
400	416·0	415	15	
	432·4	433	33	
	460·6	460	60	
373	387·2	388	15	
	495·0	496	123	$C_6H_5NO_2$
	511·8	511	138	$C_9H_{14}O$
	528·9	529	156	
346	363·6	364	18	
	387·9	388	42	
	406·9	406	60	
	469·9	469	123	
	530·1	529	183	

TLC examination of the neutral aglycone fraction from *M. rostrata* collected from the Wollongong area showed the presence of about eight constituents. One of these, separated

[12] F. SCHAUB, H. KAUFMANN, W. STOCKLIN and T. REICHSTEIN, *Helv. Chim. Acta* **51**, 738 (1968).
[13] L. SAWLEWICZ, EK. WEISS and T. REICHSTEIN, *Helv. Chim. Acta* **50**, 504 (1967).
[14] L. SAWLEWICZ, EK. WEISS and T. REICHSTEIN, *Helv. Chim. Acta* **50**, 530 (1967).

from the others by column chromatography and obtained as needles m.p. 270–272°, $[\alpha]_D^{25}$ −20°, was identified as metaplexigenin (III).[12]

EXPERIMENTAL

M. rostrata, whole plant, was collected from the Toonumbar State Forest, N.S.W. (Herbarium Voucher No. SN6615) and was recollected from the top of the escarpment at Mt. Keira, near Wollongong, N.S.W. The identification of the latter material as *M. rostrata* was confirmed by the National Herbarium, Sydney, TLC refers to chromatograms on Kieselgel G plates developed in $CHCl_3$–MeOH (9:1) and spots were visualized by spraying with H_2SO_4–Ac_2O (1:1) and warming the plates to 100°.

Isolation and identification of the constituents of the Toonumbar sample. Dried, milled plant material (4·0 kg) was extracted by percolation with cold MeOH. The extract was concentrated, acidified with H_2SO_4, diluted with H_2O and allowed to stand for 2 days. The filtered aqueous solution was basified with ammonia, extracted with $CHCl_3$ and the $CHCl_3$ extracts dried and evaporated to give the crude base mixture (18·0 g, 0·45%). A portion of the mixture (4·1 g) was submitted to chromatography on alumina using $CHCl_3$ and $CHCl_3$–MeOH as eluant.

Anabasine. Fractions showing spots which were sensitive to iodine vapour were combined and evaporated to give an oil (1·0 g). A portion of the oil was rechromatographed on preparative Kieselgel G plates developed in $CHCl_3$–MeOH (19:1) and extraction of a well defined zone yielded a brown oil $[\alpha]_D^{25}$ 0° (*c* 0·6 EtOH). Its TLC behaviour, UV., IR and MS were virtually identical with those in the literature for anabasine.[15] The picrate of the above racemate showed no depression of m.p. in a m.m.p. determination with an authentic specimen of (−)-anabasine picrate m.p. 205–207°.

Rostratine (Ia) *and dihydrorostratine* (IIa). Fractions (1·6 g) from the alumina column, which followed anabasine, showed one main blue spot of R_f 0·60. The material, consisting of *c* 2:1 mixture of Ia and IIa, crystallized from acetone to give colourless needles m.p. 264°, $[\alpha]_D^{25}$ +18 ± 2° (*c* 1·0 MeOH), λ_{max} 220, 258 (sh), 263 and 270 (sh) nm (log ϵ 3·93, 3·42, 3·45 and 3·38 resp.) (Found: C, 65·7; H, 7·5; N, 2·3; O, 23·6. $C_{29}H_{39}NO_8$ requires: C, 65·8; H, 7·4; N, 2·6; O, 24·2%; $C_{29}H_{41}NO_8$ requires: C, 65·5; H, 7·8; N, 2·6; O, 24·1%) ν_{max} 3520, 3460, 1730, 1715 and 1600 cm^{-1}. The NMR and the MS metastable mode results (peaks resulting from parent at *m/e* 529) are shown in Tables 1 and 2 respectively. The genin (1·0 g) in MeOH (50 ml) and 5 N KOH (2 ml) was refluxed for 5 hr. The mixture was diluted with H_2O, MeOH removed by distillation and the solution cooled. The colourless needles (250 mg) formed were filtered off. Repeated ether extraction of the mother liquors yielded a further 300 mg. Recrystallization of the mixture gave colourless needles m.p. 230–250° (Found: C, 66·0; H, 9·1. Calc. for $C_{21}H_{34}O_6$: C, 65·9; H, 9·0%. Calc. for $C_{21}H_{36}O_6$: C, 65·6; H, 9·5%). ν_{max} 3560, 3460, 3400 and 3340 cm^{-1} (OH). The MS showed no parent but had peaks at 364·2250 ($C_{21}H_{32}O_5$) and 366·2387 ($C_{21}H_{34}O_5$) in the ratio of about 2:1. Other prominent peaks were at *m/e* 337, 328, 320, 319, 302 and 301 and were accompanied by $p + 2$ peaks of about $\frac{1}{2}$–$\frac{1}{8}$ intensity. This mixture was dissolved in HOAc (30 ml) and the solution hydrogenated at 1 atm H_2 over Pt for 4 hr. Trituration with H_2O followed by several recrystallizations from acetone gave 5α-dihydrosarcostin (IIb) m.p. 264–266° $[\alpha]_D^{25}$ +47·5 ± 2° (lit.[13] m.p. 267–269°, $[\alpha]_D$ +50·5°) identical with (I.R. and MS T.L.C. and m.m.p.) an authentic specimen.

Mother liquors from the alkaline hydrolysis were acidified with HCl, concentrated to about 20 ml and placed on a column of Zeo-Karb 225 in H^+ form. The column was washed with H_2O and then with 2 N NH_4OH. The ammonia washings were concentrated and the pH adjusted to 4·5 by careful addition of HCl. The colourless crystals m.p. 230°, which formed were identical (m.m.p. and I.R.) with an authentic specimen of nicotinic acid.

Extraction of the Mt. Keira sample. Milled, dried plant material (4·5 kg) was extracted by percolation with cold MeOH (12 l.) and the extract concentrated to about 1·5 l. The concentrated MeOH solution was exhaustively extracted with light petrol. (b.p. 60–80°), then 0·5 l. 2 N H_2SO_4 was added and the solution was allowed to stand for 3 days. The supernatant liquor was decanted from the tar and concentrated under reduced pressure until it became turbid. It was then diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ extract washed with dil. acid, dil. $NaHCO_3$ and finally with H_2O. The dried $CHCl_3$ solution was evaporated to give the neutral aglycones as a viscous yellow oil (115 g). The aqueous acid solution and acid washings were basified with ammonia, extracted with $CHCl_3$ and the $CHCl_3$ dried and evaporated to give the bases as a brown oil (13·0 g). Column chromatography of the bases gave a mixture of rostratine and dihydrorostratine. Anabasine could not be detected. A portion of the neutral aglycone fraction was chromatographed on an alumina column using $CHCl_3$ with increasing methanol content as eluant. The fraction, which showed a

[15] A. M. DUFFIELD, H. BUDZIKIEWICZ and C. DJERASSI, *J. Am. Chem. Soc.* **87**, 2926 (1965).

single blue spot at R_f 0·61, yielded (from acetone) colourless needles of metaplexigenin (III) m.p. 270–272° $[\alpha]_D^{25}$ $-20 \pm 2°$ (*c* 0·6 MeOH),[12] identified by direct comparison with an authentic specimen.

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