pounds were revealed with either 20% $SbCl_5$ or 5% anisaldehyde in 95% ethanol–H_2SO_4, 19·1 v/v GLC was performed using a 3% OV-17 and a 1% SE-30 column, at 270° and 240° respectively Multiple analyses were made for each sample with reference samples being run before and after each sample GLC-MS was performed on a LKB Model 9000 single focusing gas chromatograph-mass spectrometer A 3% OV-17 column was used at a column temperature of 280°

A nonsaponifiable fraction was prepared from the ester fraction by refluxing it for 2 hr in a H_2O–benzene–EtOH (1/1/8, vol) solution containing 15% KOH, followed by extraction with Et_2O

Isolation of sterol glycoside mixture from *N sanguinea* The EtOH eluate (from an alumina column) yielded 300 mg of a solid melting about 290° to a dark red melt Repeated crystallization yielded a white solid, m.p 275–280°, $[\alpha]_D^{20°}$ − 44 08 (pyridine). Reported sitosteryl β-D-glycoside, m p 285°, with optical rotation at $[\alpha]_D^{20°}$ − 41 5 (pyridine) [18] (Calc for $C_{35}H_{60}$, C, 72 87, H, 10 45 (β-sitosteryl glycoside), Found. C, 72 23, H, 10 45%) Acetate formed from Ac_2O and pyridine (1 1 vol), m p 172–173°, $[\alpha]_D^{20°}$ 0° ($CHCl_3$), (Calc for $C_{43}H_{68}O_{10}$ C, 69 32, H, 9 20 (sitosterolin tetra-acetate) Found· C, 69 30, H, 9 12%)

Hydrolysis of the sterol glycoside from *N sanguinea* Refluxed (20 hr) in EtOH– HCl (5 1 vol). Mixture after dilution with H_2O, extraction with Et_2O and distillation of the latter yielded a white crystalline solid Repeated crystallization gave white crystals, m p 137–139°, acetate, m p 127–129° GLC of the free sterols indicated the presence of campesterol (5%), stigmasterol (10%), and sitosterol (85%)

Acknowledgements—We are indebted to Mr A Naqvi and Mr T Y Tok for their able assistance in this study and to Mr Chandapilla of the Rubber Research Institute of Malaysia for identification of the *Nepenthes* obtained from the Cameron Highlands area We are grateful to the following individuals for generous gifts of reference compounds· Drs J W Rowe (citrostadienol), F Knapp (28-isofucosterol acetate), A Gonzales (obtusifoliol) This work was supported by Grant GB 19113 from the National Science Foundation GLC–mass spectrometry was supported by NIH Grant AM-09992

[18] Elsevier's Encyclopedia of Organic Chemistry, *Sitosteryl Esters and Glycosides*, Series III, Vol 14, p 1820, Elsevier, Amsterdam (1954)

Key Word Index—Nepenthes albomarginata, *Nepenthes sanguinea*, Nepenthaceae, sterols, triterpenes

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PAPAVERACEAE

THE ALKALOIDS OF *ARGEMONE GRANDIFLORA*

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Abstract—The major alkaloids of the above-ground parts of *Argemone grandiflora* Sweet subsp *grandiflora* were found to be berberine, α-allocryptopine, and protopine, (+)-laudanosine, (+)-codamine, (−)cheilanthifoline, corypalmine, sanguinarine, and chelerythine were identified as minor alkaloids This species is placed in Alliance IVa of the Stermitz classification, and appears to be a phylogenetically young member of the genus

INTRODUCTION

THE ALKALOIDS of *Argemone* species are of interest on account both of their pharmacological properties, and their potential use in the systematics of the genus. Thus the discovery[1] that 'epidemic dropsy' in India was caused by contamination of cooking oils with *Argemone mexicana* L seed-oil was followed by the demonstration[2] that the alkaloid sanguinarine, present in the seed-oil, increased intra-ocular pressure and caused dropsy and glaucoma in

[1] S L SARKHAR, *Ind Med Gaz* **61**, 62 (1926), *C A* **20**, 2022 (1926)
[2] S A HAKIM, *Br J Ophthal* **38**, 193 (1954)

experimental animals Subsequently,[3] a number of other isoquinoline alkaloids were shown to possess the same property, and it has been suggested[4] that endemic glaucoma is linked to the ingestion of these alkaloids.[4,*] More recently Stermitz[6] has suggested the division of the genus *Argemone* into four alliances on the basis of the presence or absence of protopine-, berberine-, and pavine-type alkaloids, as well as on morphological features We report here the results of our investigations of the alkaloids of *A grandiflora* Sweet subsp *grandiflora* †

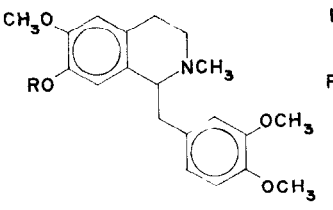
On the basis of paper chromatographic evidence, Hakim, Mijovic, and Walker[4] have previously reported that *A grandiflora* Sweet contained berberine in the capsules, and possibly in the roots, coptisine in the roots, stems, leaves, flowers, and capsule, chelerythine in the roots; and sanguinarine in the roots, flowers, and capsules, and possibly in the stems and leaves

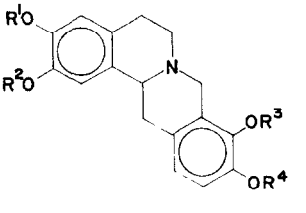
Aregemone grandiflora subsp *grandiflora*‡ was grown locally from seed collected at Zimapán, Hidalgo, Mexico After luxuriant growth, the plants were harvested when in full blossom. The aerial parts of the plants were separated from the roots and each processed for alkaloids as described in the Experimental

RESULTS

The major alkaloids of the aerial parts were protopine (IIIa), α-allocryptopine (IIIb) and berberine (IV), accounting for about 75% of the total alkaloids.

Examination of the minor alkaloids resulted in the isolation and identification of the tetrahydroisoquinolines L-(+)-codamine (Ia) and L-(+)-laudanosine (Ib), as well as trace amounts of the tetrahydroprotoberberines corypalmine (IIa), and the rarely encountered (−)cheilanthifoline (IIb) §





(Ia) R = H

(Ib) R = Me

(IIa) $R^1 = H$, $R^2 = CH_3$, $R^3 = R^4 = CH_3$

(IIb) $R = CH_3$, $R^2 = H$, $R^3 + R^4 = CH_2$

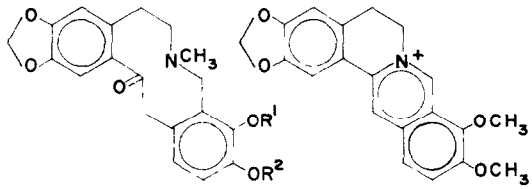
* However, it has been reported to us[5] that the alkaloid-free seed-oil of *A mexicana* is also potent in producing increased intra-ocular pressure, the activity being concentrated in the highly unsaturated, triglyceride fraction

† Formerly known as *A grandiflora* Sweet In his thorough, and most useful revision of the genus *Argemone*, Ownbey[7] recognized *A intermedia* Fedde as *A grandiflora* Sweet subsp *armata* Ownb, and renamed the historically well known *A grandiflora* Sweet as subsp *grandiflora*

‡ Our identification was confirmed by Drs R T Ogilvie, Custodian of this University's Herbarium, and G B Ownbey of the Department of Botany, University of Minnesota, Minneapolis Specimens were deposited in the Herbaria of both Universities

§ Laudanosine was recently reported by Stermitz and Coomes[8] as a minor alkaloid of *A pleiacantha* Greene subsp *pinnatisecta* Ownb, they commented that the only other reported natural occurrence of this alkaloid was in *Papaver somniferum* Codamine, corypalmine and cheilanthifoline do not appear to have been reported in *Argemone* before

[3] W A Lieb and H J Scherf, *Klin Mbl Augenheilk* **128,** 686 (1956), *Index Medicus* **60,** 958 (1956)
[4] S A Hakim, V Mijovic and J Walker, *Nature, Lond* **189,** 198 (1961)
[5] Private communication from Dr R T Aplin, Dyson Perrins Laboratory, South Parks Road, Oxford
[6] F R Stermitz, D E Nicodem, C C Wei and K O McMurtrey, *Phytochem* **8,** 615 (1969)
[7] G B Ownbey, *Mem Torrey Bot Club* **21,** 1 (1958)
[8] F R Stermitz and R M Coomes, *Phytochem* **8,** 611 (1969)



(IIIa) $R^1 + R^2 = CH_2$ (IV) (Va) $R^1 + R^2 = CH_2$

(IIIb) $R^1 = R^2 = CH_3$ (Vb) $R^1 = R^2 = CH_3$

In addition, paper and thin-layer chromatographic analysis revealed the presence of two benzophenanthridine alkaloids, sanguinarine (Va) and chelerythine (Vb), as well as traces of at least three other unidentified bases.

The major root alkaloids were also IIIa, IIIb and IV, but Ia, Ib, IIa and IIb were no, detected, while Va and Vb, present in greater amounts than in the aerial parts of the plant were actually isolated *

DISCUSSION

Apart from providing another illustration of the co-occurrence of a range of biosynthetically related benzylisoquinoline alkaloids, the results of our analysis together with the morphology of the plant, place *A grandiflora* Sweet subsp *grandiflora* within alliance IVa of Stermitz's system with the implication[6,10] that it is a phylogenetically young member of the genus

EXPERIMENTAL

The alkaloids were separated by standard procedures from exhaustive methanol extracts of fresh, and air-dried plant material [11] In outline, the methanol extracts were concentrated, and then partitioned between light petroleum (b p 40–60°) and aq H_2SO_4, the aqueous phase was washed with ether, brought to pH *ca* 10 and extracted with ether until it no longer gave a positive reaction with Mayer's, or Wagner's reagents, evaporation of the combined, dried, ether extracts then gave the crude mixed alkaloids, which amounted to 0 53% wt /dry wt in the case of the air-dried above-ground plant The alkaloid mixture was then separated by conventional procedures, including division into phenolic- and non-phenolic bases, fractional crystallization and, in the cases of the minor alkaloids, PLC In the first instance identifications were made by spectroscopic measurements, especially the mass spectral fragmentation patterns which are so characteristic for these groups of alkaloids, and subsequently confirmed by comparison of the alkaloids with authentic specimens We record below only MS and PMR data for (−)-cheilanthifoline lacking in the literature The approximate alkaloid composition (expressed as per cent of crystalline alkaloids isolated from the mixed bases from the above-ground plant parts) was as follows protopine (40), α-allocryptopine (25), berberine (7),

* Incidentally, some species of *Argemone*, among which is *A grandiflora* subsp *grandiflora*, are characterized by having a bright yellow latex, and Stermitz has suggested that this colour is due to berberine [9] We examined both leaf-, and root-latex by paper chromatography[4] and found that the yellow pigment was berberine, on the basis of its having the same R_f as berberine, exhibiting the same brilliant lemon-yellow fluorescence in UV light and giving a positive reaction with Dragendorff reagent

[9] F R STERMITZ, in *Recent Advances in Phytochemistry* (edited by T J MABRY, R E ALSTON and V. C RUNECKLES), Vol 1 Appleton–Century–Crofts, New York (1968)

[10] F. R STERMITZ, S M WORKMAN and W M KLEIN, *Phytochem* **10,** 675 (1971)

[11] F R STERMITZ and J N SEIBER, *J Org Chem* **31,** 2925 (1966)

L-(+)-codamine (4), L-(+)-laudanosine (2), (−)-cheilanthifoline (0·1), corypalmine (trace), sanguinarine (trace, 0·5 in root alkaloids), chelerythine (trace).

(−)-*Cheilanthifoline*. *m/e* 325·1320 (M^+, calc. for $C_{19}H_{19}NO_4$ 325·1314) (41), 324 (25), 176 (12), 148 (100); τ($CDCl_3$) 6·13(3H,s, OCH_3) 6·50 and 5·87 (2H,AB q, J = 16 Hz, H-8), 4·06(2H,s, OCH_2O), 3·40, 3·34 and 3·17 (4H, m, aromatic H).

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Key Word Index—Papareraceae; isoquinoline alkaloids; protopine; allocryptopine; berberine.

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PEDALIACEAE

FLAVONOIDS OF THE LEAVES OF *PEDALIUM MUREX*

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Plant. Pedalium murex L. *Uses.* Medicinal.[1] *Previous work.* Alkaloid and fatty oil from fruits.[1]

Present work. Fresh leaves extracted with 80% alcohol by hot refluxing. The aq. concentrate fractioned into petrol (40–60°), ether, ethyl acetate solubles and the aqueous mother-liquor.

Ether ext. Pedalitin (6-methoxy-5,7,3′,4′-tetrahydroxyflavone) (m.p. and mixed m.p., acetyl, m.p. and mixed m.p., R_f and co-chromatography with authentic sample from *Sesamum indicum*[2]), diosmetin and dinatin (6-methoxy-5,7,4′-trihydroxyflavone) (co-chromatography and direct comparison with authentic samples) (Table 1).

TABLE 1. R_f OF THE FLAVONOIDS OF *Pedalium murex*

Compound	R_f (Whatman No. 1, ascending, 28 ± 2°)					
	H_2O	15% HOAc	30% HOAc	BAW	Forestal	H_2O satd. phenol
Pedalitin	0·00	0·06	0·24	0·67	0·45	0·83
Dinatin	0·02	0·12	0·36	0·95	0·85	0·93
Diosmetin	0·00	0·07	0·22	0·90	0·76	0·85
Pedaliin	0·07	0·24	0·52	0·43	0·76	0·75
Dinatin-7-glucuronide	0·45	0·30	0·53	0·63	0·80	0·60
Diosmetin-7-glucuronide	0·60	0·21	0·42	0·46	0·65	0·46

[1] *Wealth of India, Raw Materials*, Vol. VII, p. 284. C.S.I.R., New Delhi (1966).

[2] N. R. KRISHNASWAMY, T. R. SESHADRI and P. J. TAHIR, *Indian J. Chem.* **8**, 1074 (1970).