Phytochemistry, 1975, Vol. 14, pp. 1785–1788. Pergamon Press. Printed in England.

ALKALOIDAL CONTENT OF ARGENTINE *ARGEMONE**

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(*Received* 4 *January* 1975)

Key Word Index—*Argemone*; Papaveraceae; alkaloids; protopine; allocryptopine; berberine; sanguinarine; chelerythrine; *N*-norchelerythrine; chemotaxonomy.

Abstract—The alkaloid content of different Argentine *Argemone* has been determined. Two varieties of *A. subfusiformis* subsp. *subfusiformis* Ownb. and *A. subfusiformis* subsp. *subinermis* Ownb. yielded a similar ratio and content of the following alkaloids: protopine, allocrytopine, berberine, sanguinarine, and chelerythrine. The *A. subfusiformis* taxa showed a markedly high sanguinarine content in roots as opposed to aerial parts. *A. polyanthemos* (Fedde) Ownb. showed a different ratio between alkaloids but a qualitative similar result. *N*-Norchelerythrine was isolated from *A. polyanthemos*. The chemotaxonomic value of the alkaloid analyses is discussed.

INTRODUCTION

In our previous paper [1] we reported the identification of protopine, allocryptopine, berberine, sanguinarine and chelerythrine in the aerial parts (without capsules) of *Argemone subfusiformis* subsp. *subfusiformis* Ownb. Ownbey has described [2] an additional taxon, *A. subfusiformis* subsp. *subinermis*, as being unique to Argentina. In the Ownbey taxonomic key [2] to the *Argemone* of South America, the petal color is used as an important characteristic to separate *Argemone* of Argentina (yellow petals) from three Chilean species (white or lavender petals). However, there has been a report [3] of one white-petaled *Argemone* taxon in Argentina and in the course of our present study others have been encountered. It is evident that a closer chemical and botanical examination of South American *Argemone* is needed. As a result of detailed studies [4] of North American *Argemone*, various species have been grouped in alliances and evolutionary developments in the genus have been suggested. However, most of the reported work [4] has dealt only with analysis of aerial plant parts and there is at least one indication [5] that root alkaloid composition is quite different from aerial part composition in this genus. Such differences could possibly affect assignments to alliances. We have for these reasons undertaken a detailed look at the alkaloid content of additional Argentine *Argemone* taxa.

RESULTS

Table 1 shows the results of the analyses conducted.

A. subfusiformis

Extensions of the previous work [1] on subsp. *subfusiformis* were conducted. In the first case, a white petaled variety of subsp. *subfusiformis* was investigated. This was discovered near locations of the "normal" yellow-petaled variety and had some morphological variations other than petal color [6]. Chemically it proved essentially indistinguishable from the yellow-petaled subsp. *subfusiformis*. Roots of subsp. *subfusiformis* were investigated and showed a remarkable change in sanguinarine concentration, which now became the major alkaloid. The fresh stem latex of subsp. *subfusiformis* is bright yellow when first cut, but

* Part 5 in the series *Alkaloids of Argentine Medicinal Plants*. For part 4, see Ref. [4].

Table 1. Alkaloid content of some Argentine *Argemone* taxa

	A. subfusiformis subsp. *subfusiformis*				*A. subfusiformis* subsp. *subinermis*		*A. polyanthemos*	
	Yellow Petalled		White Petalled					
	Aerial parts* (%)	Roots (%)	Aerial parts (%)	Roots (%)	Aerial parts (%)	Roots (%)	Aerial parts (%)	Roots (%)
Protopine	41	26	55	28	46	20	trace	trace
Allocryptopine	28	18	34	17	31	17	50	40
Berberine	9	7	8	19	16	12	12	15
Sanguinarine	5	47	3	3	4	42	trace	trace
Chelerythrine	4	1		3		2	15	15
Other	13			9	trace	7	30†	30†
Total alkaloids as % dry weight	0·4	1·4	0·8	0·4	0·6	0·9	0·8	0·8

* Capsule content was measured separately from other aerial parts and was found to contain the same alkaloid ratios.
† The major component was *N*-norchelerythrine.

changes to orange upon contact with the air. Root cuttings are also yellow, but become dark orange in air; root slices show yellow fluorescence when fresh, but red fluorescence upon aging. These findings are consistent with the suggestion that sanguinarine is a minor or even negligible natural plant component in this species, but that it is formed rapidly upon exposure to air after injury. The true component is probably dihydrosanguinarine. The original yellow color of the latex is undoubtedly due to the berberine and chelerythrine content.

Interesting petal color changes were also observed in the case of the white-petaled variety of subsp. *subfusiformis*. The originally white petals became yellow several days after cutting. Preliminary paper chromatography tests showed the presence of berberine and sanguinarine in the aged white petals from subsp. *subfusiformis*, while only berberine was present in the aged petals of the variety whose natural growing petal color was yellow.

Collections of *A. subfusiformis* subsp. *subinermis* were made and investigated chemically. Although the morphological differences between the subspecies are apparent and the subspecies are geographically not contiguous, there were no chemical differences found. The subsp. *subinermis* also exhibited a very high root content of sanguinarine.

A. polyanthemos

One collection of *Argemone* species was made in southern Córdoba province where *A. subfusiformis* subsp. *subfusiformis* was growing contiguously with another taxon which was identified [7] as *A. polyanthemos*. The *subfusiformis* grows independently throughout central Argentina, but this is the only known location for *A. polyanthemos*, a species native to the west and central United States. Locations in east Texas and Utah have been viewed [8] as introductions. Although species such as *A. mexicana* and *A. ochroleuca* are found as introductions in harbors throughout the world, the finding of *A. polyanthemos* in the central pampa area of Argentina is unique. Although *A. polyanthemos* had been investigated previously [4], the plant from the Argentina location was analyzed and found to contain considerable amounts of *N*-norchelerythrine and chelerythrine which had not been previously identified from this species; the major alkaloid was allocryptopine. The earlier work [4] had also indicated berberine as the major alkaloid. Since isolation techniques were different, we re-examined the alkaloid content of the United States collection and found the reported [4] 80% berberine, 15% allocryptopine content to be essentially correct. Through TLC of minor fractions, we have detected the presence of chelerythrine and *N*-norchelerythrine, although only in trace amounts.

There is an indication in the literature [9] that benzophenanthridinium alkaloids may not be stable in solution and that at least one demethylates easily. Since *N*-norbenzophenanthridinium alkaloids have recently been reported from several species, we investigated briefly the stability of chelerythrine in solution under varying conditions but could find no conversion to *N*-norchelerythrine in detectable amounts. The *N*-norchelerythrine is therefore not an artifact produced during isolation.

DISCUSSION

In terms of chemistry, the most interesting observations were the discovery of a large sanguinarine content in roots of *A. subfusiformis* taxa as compared to the aerial parts, the fact that such a variation does not occur in *A. polyanthemos* and the discovery of *N*-norchelerythrine in *A. polyanthemos*. There have not been sufficient root alkaloid analyses performed in *Argemone* for the chemotaxonomic significance (if any) of sanguinarine accumulation in roots to be assessed. However, both *A. mexicana* [10] and *A. albiflora* [11] have had separate analyses of roots and aerial parts. These species did not show the marked increase in sanguinarine content which we have here observed with *A. subfusiformis*, even though the general chemical content and morphology indicates that *A. subfusiformis* should be closely related to *A. mexicana* and *A. albiflora* (all of Alliance IV [4]). *N*-Norchelerythrine (along with *N*-norsanguinarine) were recently reported [11] to be minor constituents of *A. albiflora*. It has very recently been shown [12] that benzophenanthridine alkaloids (including the *N*-nor derivatives) are accumulated by callus tissues of various poppy species even though these may not be present in the normal plant. Indeed, callus tissue alkaloids from 11 species of Papaveraceae were found [12] to be nearly identical even though the redifferentiated plantlets derivable from the callus tissue again showed the typical varying alkaloid content of the normal plant. These experiments suggest that benzophenanthridine alkaloids may not be significant chemotaxonomic markers.

Because of the variability and instability of petal color which we have found in the Argentine *Argemone* species, the systematic key [2] to the genera of South America will need modification.

EXPERIMENTAL

Plant material. Capsules and roots of *Argemone subfusiformis* subsp. *subfusiformis* Ownb. (yellow petals) collected in the Province of Córdoba (Argentina) were used. A voucher specimen is deposited in the Museo de Botánica, Universidad Nacional de Córdoba, Ariza 2553 (CORD). Capsules came from nearly 200 specimens. Roots came from at least 50 specimens. *A. subfusiformis* subsp. *subfusiformis* Ownb. (white petals) was collected in the Prov. of Córdoba, Argentina (voucher specimen 71 of our herbarium). *A. subfusiformis* subsp. *subinermis* Ownb. was collected in the Prov. of Entre Ríos (Argentina) and a voucher specimen is deposited in our herbarium (no. 67). *A. polyanthemos* (Fedde) Ownb. was collected in Huinca Renancó, Prov. of Córdoba, Argentina (voucher specimen 16 of our herbarium).

Extraction. The capsules of *A. subfusiformis* subsp. *subfusiformis* (yellow petals) (1040 g) were defatted with *n*-hexane, extracted exhaustively with 95% EtOH and this extract treated and their alkaloids isolated as previously described [1].

The alkaloids of the remaining plants were extracted as described previously [1], except for the aerial parts of *A. polyanthemos* that, for isolation purposes, was as follows: The dried sample (1000 g) was extracted with 95% EtOH in a Soxhlet and concentrated to a final vol of 2 l. This extract was diluted to 4 l. with 1% H_2SO_4, filtered and concentrated to 2 l. This filtrate was extracted successively in a separating funnel with different solvents [petroleum ether (fraction I), Bz (II), ether (III), $CHCl_3$-*i*-PrOH (3:1) (IV)]. The aq. soln was made alkaline (pH = 10–11) with 20% NaOH and extracted with the same series of solvents (fractions Ia–IVa). Then it was neutralized (pH = 6–7) with H_2SO_4, made alkaline (pH = 8–9) with conc NH_3, and extracted again with the same series of organic solvents (fractions Ib–IVb). Through concentration of the corresponding fractions the following alkaloids were isolated, recrystallized and identified by comparison with authentic samples (mp, mmp, TLC, UV, IR, NMR): chelerythrine (II), allocryptopine (Ia, IIa, IIIa), berberine and allocryptopine (IIb, IIIb).

Isolation of N-*norchelerythrine.* Once the chelerythrine was isolated, the mother liquor of fraction II was concentrated to dryness. The gummy residue was washed successively with Et_2O and H_2O. The insoluble fraction was crystallized from 95% EtOH, giving 109 mg needles (blue fluorescence in EtOH, green fluorescence in $CHCl_3$), mp: 211–213° (EtOH) (lit. [11] mp 213–214°). UV, IR and mass spectra were identical with those reported [11]. This product was identical (TLC, 3 solvents, mp, mmp, UV) with *N*-norchelerythrine that was prepared by sublimation (200°, 0·05 mm Hg) of 2 mg chelerythrine [13].

Chelerythrine was dissolved in aqueous EtOH and allowed to stand 24 hr, then heated for 48 hr at reflux. Samples were withdrawn at various times for TLC analysis, but no *N*-norchelerythrine was found.

Samples of *A. polyanthemos* plant material from the United States [4] were subjected to the isolation scheme described above. Because of the high berberine content, this alkaloid appeared in fractions I, IV and IVa as well as those listed above for the Argentine collection. Traces of *N*-norchelerythrine were found in fraction II and chelerythrine in IV, Ia and IIa.

Acknowledgements—The authors are indebted to Dr. B. S. Sorarú for botanical advice, to M. E. Mendiondo for preliminary analysis of petals, to M. Quante for rechecking the United States *A. polyanthemos* content and to T. A. Stermitz for the experiments on chelerythrine stability. This work was supported in part by a Research Grant of CONYCET No. 3520c/73, a Research Grant of the Universidad de Buenos Aires, Expte. no. 24174/71, and a Senior Fulbright-Hays Award to F.R.S.

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