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ALKALOIDS OF *ARGEMONE POLYANTHEMOS*, *A. CORYMBOSA*, *A. CHISOSENSIS*, *A. SANGUINEA*, *A. AURANTIACA* AND GENERAL *ARGEMONE* SYSTEMATICS[1]

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Abstract—The alkaloid contents of *Argemone polyanthemos* (Fedde) Ownb., *A. corymbosa* Greene subsp. *arenicola* Ownb., *A. chisosensis* Ownb., *A. sanguinea* Greene, and *A. aurantiaca* Ownb. were determined. Phylogenetic relationships within the genus *Argemone* and among *Argemone* and other genera are discussed.

AS RECENTLY revised[2] by Ownbey, the genus *Argemone* is composed of twenty-eight species, with a number of subspecies and varieties also being recognized. By 1963, a total of seven species had been analyzed for alkaloids and the suggestion was made[3] that the genus seemed to consist mainly of two groupings depending upon the presence or absence of the pavine-type (I) alkaloids. A single species, *A. platyceras*, contained both pavine-type alkaloids and major amounts of others and was therefore considered[3] an intermediate case. By 1966 a number of additional species had been investigated and we were able to point out[4] that our preliminary studies showed a continued correspondence with the suggested[3] groupings and that, moreover, these groupings corresponded in most instances to some "poorly defined alliances" suggested[2a] by Ownbey on the basis of morphology. In the present paper we wish to report on the alkaloid content of an additional five species, to summarize the total available data (now comprising fifteen of the twenty-eight described species), and to amplify and modify our preliminary[4] suggestions on the relationships among the species of *Argemone*.

RESULTS

Argemone polyanthemos (*Fedde*) *Ownb.*

This species is common in the western United States from Wyoming to Texas, with one (presumably introduced) isolated occurrence in south central New Mexico. The plant was known throughout the botanical literature as *A. intermedia* Sweet prior to Ownbey's revision.[2a] We investigated two populations: one from eastern Wyoming and the other from

[1] Part XII in the series "Alkaloids of the Papaveraceae"; for Part XI, see F. R. STERMITZ and R. M. COOMES, *Phytochem.* **8**, 611 (1969). The present work was supported by Grant GM-15424 from the U.S. Public Health Service.

[2] (a) G. B. OWNBEY, Monograph of the Genus Argemone for North America and the West Indies, Memoirs of the Torrey Botanical Club, Vol. 21, The Seeman Printery, Durham, North Carolina (1958). (b) G. B. OWNBEY, *Brittonia* **13**, 91 (1961).

[3] J. SLAVIK and L. SLAVIKOVA, *Coll. Czech. Chem. Commun.* **28**, 1728 (1963).

[4] F. R. STERMITZ, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY), Vol. 1, Chap. 5, Appleton-Century-Crofts, New York (1968). This is the published report of a symposium held in Austin, Texas, in 1966.

the isolated New Mexico occurrence. Both were very similar in alkaloid content, containing about 0·1 per cent total alkaloids of which berberine represented the major alkaloid, with allocryptopine in minor quantities and protopine and sanguinarine identified as traces.

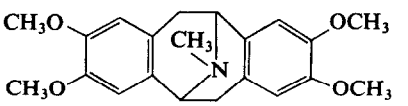
A. corymbosa *Greene subsp.* arenicola

Plants of this subspecies were obtained from its relatively restricted occurrence in southern Utah and northern Arizona. Berberine was the major alkaloid, allocryptopine and protopine

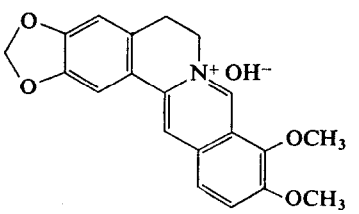
TABLE 1. ALKALOIDS OF SOME SELECTED *Argemone* SPECIES

Species	Total alkaloid content[a] (%)	Per cent of total alkaloids						
		I	II	IIIa	IIIb	IIIc	IV	V
A. polyanthemos (New Mexico)	0·12	—	82	17	*T*[b]	—	*T*	—
A. polyanthemos (Wyoming)	0·07	—	86	13	*T*	—	*T*	—
A. corymbosa subsp. *arenicola*	0·09	—	92	4	3	—	*T*	—
A. chisosensis	0·04	—	88	11	*T*	—	—	—
A. sanguinea (purple flowered)	0·07	—	94	—	—	6	—	—
A. sanguinea (white flowered)	0·05	6	68	22	—	4	—	—
A. aurantiaca	0·10	—	—	—	60	—	—	40

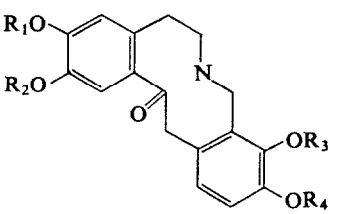
(a) Per cent by weight of dried plant materials. (b) *T*=trace.



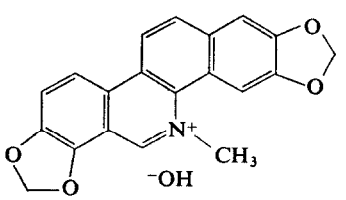
(I) Argemonine



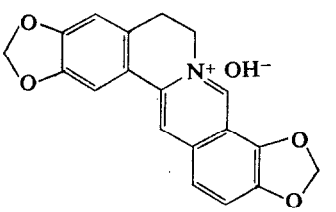
(II) Berberine



(IIIa) Allocryptopine ($R_1 + R_2 = —CH_2—$; $R_3 = R_4 = CH_3$)
(IIIb) Protopine ($R_1 + R_2 = R_3 + R_4 = —CH_2—$)
(IIIc) Muramine ($R_1 = R_2 = R_3 = R_4 = CH_3$)



(IV) Sanguinarine



(V) Coptisine

were present in minor amounts and a trace of sanguinarine was identified. We have so far failed in attempts to find *A. corymbosa* subsp. *corymbosa*, which is reported to occur chiefly in the Mohave desert of California.

A. chisosensis *Ownb.*

This species of south-western Texas was collected near Big Bend National Park. It is similar to *A. sanguinea* Greene and was accorded species rank by Ownbey "with some hesitancy". Again, berberine proved to be the major alkaloid while allocryptopine was found in minor quantities along with traces of protopine.

A. sanguinea *Greene*

This species occurs throughout southern Texas and adjacent Mexico. Ownbey reported[2a] that his treatment of the species should be considered preliminary because of the large degree of morphological variation which he included under this binomial. We investigated two populations showing a degree of floral variation. One population contained the typical large amount of the major alkaloid berberine, but the only other alkaloid we detected was a minor amount of muramine. The second population again contained berberine as the major alkaloid (although somewhat decreased in concentration) along with rather more allocryptopine than was present in the species described previously. A few per cent each of muramine and (surprisingly) argemonine were identified.

A. aurantiaca *Ownb.*

This interesting species (morphologically unrelated to any of the above) occurs in relatively restricted locations of the hill country of south central Texas. Our collection (as opposed to our general practice) was unfortunately composed mainly of plants in the rosette stage and therefore the results reported here can only be considered preliminary with regard to this species. The two major alkaloids found were coptisine and protopine. This represents a deviation from the typical high content of berberine and allocryptopine in the rest of the Texas *Argemone*.

For the purposes of discussion, Table 1 summarizes the above data.

DISCUSSION

The alkaloid contents of *Argemone polyanthemos, A. corymbosa* subsp. *arenicola*, and *A. chisosensis* are essentially identical even though there are large differences in morphology among the three species. This identity of alkaloid content among *species* is magnified when one sees the obvious difference in two *populations* of *A. sanguinea* or the differences among many populations of *A. pleiacantha*.[1] Thus, alkaloid content has failed to distinguish between the first three species of Table 1. The differences between *A. chisosensis* and *A. sanguinea*, however, indicate that Ownbey's[2a] "hesitant" separation of the two may have been correct. The differences between the two populations of *A. sanguinea* complement Ownbey's observation[2a] of large morphological differences among the members of this species and indicate that a further systematic investigation of *A. sanguinea*, both morphologically and chemically, would be of value. The finding of a small per cent of argemonine in *A. sanguinea* is of interest. In view of the morphological closeness of *A. sanguinea* to most of the other Texas *Argemone* (all of which lack this alkaloid), we do not view the occurrence of argemonine as damaging

to our general system. It is abundantly evident that *A. sanguinea* exhibits wide genetic variation and we would explain the occurrence of argemonine as a "reconstruction of an ancestral biosynthetic pathway" as did M. Ownbey in a similar case[5] involving chemotaxonomy of some *Dicentra* species and hybrids.

Although he commented on some similarities to *A. hispida*, Ownbey considered[2a] *A. aurantiaca* to probably be most closely related to *A. squarrosa*, whose range is directly west of that of *A. aurantiaca*. Although our results are still preliminary on *A. aurantiaca*, its alkaloid content is obviously not similar to that of *A. hispida*. Coptisine was identified for the first time in the *Argemone* in large concentrations. Previously it had been found that *A. squarrosa* subsp. *squarrosa* contained mainly allocryptopine[6] or berberine[7] and muramine[7] and hence, although the alkaloids of *A. aurantiaca* and *A. squarrosa* are not exactly the same, they are nevertheless closely related biosynthetically.

SYSTEMATICS OF THE GENUS *ARGEMONE*

The preliminary suggestion[4] of alliances among the *Argemone* can now be extended by the present and other intervening work. Table 2 lists our present groupings for the fifteen species whose alkaloid content is now known.

TABLE 2. ALLIANCES IN THE GENUS *Argemone*

I	II	III	IVa	IVb
A. hispida	*A. platyceras*	*A. squarrosa*	*A. albiflora*	*A. mexicana*
A. munita	*A. pleiacantha*	*A. aurantiaca*	*A. polyanthemos*	*A. ochroleuca*
A. gracilenta			*A. corymbosa*	*A. aenea*
			A. sanguinea	
			A. chisosensis	

In Alliance I are placed the species which concentrate argemonine-type alkaloids. In each of the species, these alkaloids represent over 90 per cent of the total alkaloid content. Traces of the protopine-types and tetrahydrobenzylisoquinolines occur. We have not found berberine in any of these three species and this is in accord with their general descriptions[2a] and our observations of a latex which is a pale lemon yellow color. Ownbey has reported that both *A. munita* and *A. gracilenta* in rare cases show a more intense yellow latex, which may point to the presence of berberine in some instances.

Alliance II represents the intermediate species which either have an approximately equal amount of argemonine-type and protopine-type alkaloids (*A. platyceras*[3]), or, in the case of *A. pleiacantha*,[1] have populations which vary from almost complete lack of the former to almost complete lack of the latter.

Alliance III contains the two highest polyploids of the genus, which, aside from that factor and alkaloid content, bear closest morphological relationships to *A. hispida*. Ownbey reported[2a] chromosome numbers for seventeen of the twenty-three North American *Argemone*. The basic number of the genus is thought[2a, 8] to be 7, but no species with a meiotic

[5] D. FAHSELT and M. OWNBEY, *Am. J. Botany* **55**, 334 (1968).
[6] T. O. SOINE and R. E. WILLETTE, *J. Am. Pharm. Assoc., Sci. Ed.* **49**, 368 (1960).
[7] F. R. STERMITZ, *J. Pharm. Sci.* **56**, 760 (1967).
[8] T. SUGIURA, *Cytologia* **10**, 558 (1940).

number of 7 has been found. Of the seventeen investigated, most had meiotic numbers of 14. *A. corymbosa* subsp. *arenicola*, *A. grandiflora*, and *A. ochroleuca* were tetraploid ($n=28$), while *A. arida* and *A. sanguinea* showed both diploid and tetraploid numbers. *A. aurantiaca* showed $2n=84$ and *A. squarrosa* subsp. *squarrosa* had $2n=ca.$ 112. Because of the complete lack of argemonine-type alkaloids in *A. squarrosa* and *A. aurantiaca*, we have set them aside from Alliance I and II. Morphology separates them from Alliance IV.

Alkaloid content sets Alliance IV aside from the other species, but it is not able to distinguish among members of the alliance. Some rather large morphological differences are present and these are partially accounted for in our division of Alliance IV in two parts. The greatest anomaly in Alliance IVa is the inclusion of *A. corymbosa*. Although a number of early workers thought *A. corymbosa* and *A. polyanthemos* to be closely related, we believe with Ownbey[2a] that *A. corymbosa* has no particular close relatives among the present *Argemone*.

PHYLOGENETIC AND PREDICTIVE VALUE

Our interest in the systematics of the genus *Argemone* has had a two-fold goal: (1) to establish phylogenetic relationships and (2) to identify new sources of the pavine-type alkaloids, which have interesting pharmacological activity.[9]

We suggested[4] that the species of Alliance I represented the oldest of the genus and hence that the presence of pavine-type alkaloids would mark the relatively more ancient members of the genus. The chief additions to the previous studies[4] have been those of the present work and the extensive study[1] of populations of *A. pleiacantha*. Morphologically, the species of the present work could have all been assigned with some assurance to the relatively more recent members of the genus and this has now been confirmed by the almost complete lack of pavine-type alkaloids in these species. The extensive investigations of *A. pleiacantha* have emphasized its intermediate placement. Thus, the geographically isolated *A. pleiacantha* subsp. *pinnatisecta* is chemically indistinguishable from the species of Alliance I, while two populations of *A. pleiacantha* subsp. *pleiacantha* were quite similar in alkaloid content to *A. sanguinea* of Alliance IVa. Thus, the phylogenetic sequence of I → II → IV is again suggested. The exact branching-off point of Alliance III is not indicated by any of our data. However, the morphological closeness and geographical distribution of this alliance when compared to *A. hispida* makes that species a prime suspect for the progenitor of Alliance III. In various phylogenetic schemes,[8,10] the genera *Argemone* and *Papaver* are considered to be the most recent developments of the family Papaveraceae. The karylogical evidence[8] seems particularly strong and suggests a late development of these two genera (both with basic chromosome numbers of 7) from a union of two main early branches: the Corydaloideae (with a basic number of 4 and containing genera such as *Dicentra* and *Corydalis*) and the Chelidonioideae (with a basic number of 3 and containing such genera as *Platystemon, Eschscholtzia, Chelidonium*, and *Glaucium*). It is interesting that the only occurrence[11] of pavine-type alkaloids so far reported in the Papaveraceae outside of *Argemone* is in *Eschscholtzia*. *Eschscholtzia* has been considered[8,10] as nearly the oldest of the Papaveraceae genera and the tie between this genus and *Argemone* (by virtue of common occurrence of pavine-type alkaloids) might either mean a closer phylogenetic relationship than has been

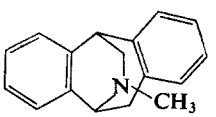
[9] Both pain relieving and heart arrythmia arresting properties have been found.

[10] F. FEDDE, in *Das Pflanzenreich* (edited by A. ENGLER), Vol. 40, W. Englemann, Leipzig; F. FEDDF, in *Die naturlichen Pflanzenfamilien* (edited by A. ENGLER and K. PRANTL), 2nd Ed., Vol. 17b (1936).

[11] R. H. F. MANSKE, K. H. SHIN, A. R. BATTERSBY and D. F. SHAW, *Can. J. Chem.* **43**, 2180 (1965).

considered previously or that the combination of genes resulting in pavine alkaloid production is only rarely observable. In this regard, it is to be noted that pavine-type alkaloids have now been reported[12] in one case outside the family Papaveraceae. That occurrence is in the Lauraceae (*Cryptocarya chinensis*), a family related to, but more ancient than, the Papaveraceae.

In the preliminary report[4] we compared the alkaloid contents of *Papaver* with *Argemone* and showed that there was little similarity in the major alkaloids of most of the species. However, one might expect that the oldest *Argemone* species might have some more close relationship to the oldest *Papaver* since they are thought[8, 10] to have arisen similarly at approximately the same time. The only section of *Papaver* which might be considered to have alkaloids similar to the pavine-type (characteristic of the oldest *Argemone*) is the section Scapiflora, whose members (such as *P. nudicaule*) are unique in the *Papaver* in that they contain[13] the isopavine alkaloids (VI). As was pointed out,[4] the section Scapiflora may well be one of the oldest of the *Papaver* and hence, the occurrence of VI may indeed indicate a closer relationship of the early *Argemone* to section Scapiflora than to other sections of *Papaver*.



(VI)

Finally, as regards our second goal (the search for additional plants containing the pavine-type alkaloids), we believe that on the basis of the results reported here we can predict which of the remaining thirteen species of *Argemone* are likely to contain major amounts of these alkaloids. If Ownbey's morphological descriptions[2] are examined carefully (using the criteria of closeness to the species of Alliances I and II), we would predict a resonable likelihood for obtaining pavine-type alkaloids from the following species: *A. fruticosa* Thurb. ex Gray, *A. subintegrifolia* Ownb., *A. echinata* Ownb., and *A. brevicornuta* Ownb.

Of these, *A. fruticosa* stands out as an important species for investigation. Of all the species, this represents the only one which Ownbey considered[2a] might possibly be removed from the genus. A number of features suggest to us that *A. fruticosa* might represent the oldest of the *Argemone* and hence its alkaloid content and, particularly, chromosome count might prove enlightening.

EXPERIMENTAL

Plant collections for the present work were made at locations described by Ownbey[2a] and are detailed below. Voucher samples of typical plants in each population were obtained. From 400 to 2000 g of dried above-ground plant material was used for each isolation. This provided sufficient pure alkaloids so that most could be determined by comparison of the isolated alkaloid with a known in terms of i.r., NMR, and mass spectra. In the case of a few trace occurrences, identity was established by comparison of the mass spectrum with the known alkaloid, as well as comparison of TLC R_f values in three solvent systems. The details of the isolation, purification, and characterization techniques have been described previously.[7, 14, 15]

[12] S.-T. LU and P.-K. LAN, *Yakugaku Zasshi* **86**, 177 (1966).
[13] F. SANTAVY, M. MATUROVA, and L. HRUBAN, *Chem. Comm. (Lond.)* 36 (1966).
[14] F. R. STERMITZ and J. N. SEIBER, *J. Org. Chem.* **31**, 2925 (1966); J. N. SEIBER, Ph.D. Thesis, Utah State University (1966).
[15] F. R. STERMITZ and K. D. MCMURTREY, *J. Org. Chem.* **34**, in press (1969); K. D. MCMURTREY, Ph.D. Thesis, Colorado State University (1968).

COLLECTIONS

Species	*Location*	*Herbarium No.*	*Remarks*
A. polyanthemos (Fedde) Ownb.	1 mi. N. of Wheatland, Wyo., on U.S. Hwy. 87	FRS 10-46327 (Colorado State)	Flowers, buds, capsules present
A. polyanthemos (Fedde) Ownb.	19 mi. E. of Las Cruces, N.M., on U.S. Hwy. 70	FRS 28-111158 (Utah State)	Flowers, buds, capsules present
A. corymbosa Greene subsp. *arenicola* Ownb.	14 mi. N. of The Gap, Ariz., on U.S. Hwy. 89	FRS 5-107940 (Utah State)	Flowers, buds, capsules present
A. chisosensis Ownb.	24 mi. S. of Marathon, Tex., on U.S. Hwy, 385	FRS 14-46337 (Colorado State)	Flowers, buds, capsules present
A. sanguinea Greene	1 mi. N.W. of Devine, Tex. (Medina Co.), on Tex. Hwy. 173	FRS 17-46332 (Colorado State)	White flowers with red–brown anthers
A. sanguinea Greene	12 mi. W. of Floresville, Tex. (Wilson Co.), on Tex. Hwy. 97	FRS 19-46330 (Colorado State)	Red–violet flowers
A. aurantiaca Ownb.	6 mi. E. of San Marcos, Tex. (Hays Co.), on U.S. Interstate 35	FRS 21-6336 (Colorado State)	Most in rosette stage, few buds, no flowers