the plastic sample container was allowed to equilibrate in a tightly closed glass receptacle and the enclosed atmosphere was analyzed.

EXPERIMENTAL

Polyethylene, polyvinyl chloride, and glass bottles were each filled with a 0.0002% v/v guarana–water solution, were separately placed in glass jars with aluminum screw caps, and stored at room temperature for at least 24 hr. A 2.0-ml. aliquot of the air space in each case was withdrawn with a Hamilton gas syringe after piercing through the metal cap with a small nail. This air sample was injected into the F & M model 609 flame ionization gas chromatograph. The small opening would always be sealed with silicone grease immediately after withdrawal of the sample. This sealing technique proved very satisfactory. The results are shown in Fig. 1. The analysis was carried out using a column of copper 10 ft. in length, 1/4 in. o.d., packed with 10% Carbowax 20M on 60–80 mesh Diatoport W. The column temperature was kept isothermally at 90° after an overnight equilibration. The injection port was 200° while the detector block temperature was 130°.

Nitrogen was used as the carrier gas with a flow rate of 45 ml./min. Hydrogen and air flow were kept at 45 and 400 ml./min., respectively. The attenuation was as indicated in Fig. 1; the range was ×1 in each case. The recorder speed was set at 15 in./hr. Each determination required approximately 30 min.

DISCUSSION

This method was found to be effective and simple. Some precautions, however, had to be employed. New septums had to be used on each day's run of the GC since the punctures made by the No. 22 gauge needle would, after a day's use, severely damage the septum and create loss in sensitivity. The cap in each sample bottle had to be kept well sealed and care had to be taken when filling the sample bottles so as not to spill any of the solution on the neck or outside surface of the bottles. The bottles were always thoroughly washed.

The column had to be conditioned overnight to give linear base at the range of ×1.

CONCLUSION

At the conclusion of the experiment the plastic containers were removed from the jars, and the containers thoroughly washed. After this washing there was still a strong odor of the guarana flavor in the air inside the plastic containers. This odor was not observed in the glass jars used in the experiment. Samples of air were withdrawn from the interior of the washed plastic containers and injected into the gas chromatograph. At an attenuation of ×32 the characteristic peaks of guarana flavor were present. Figure 1, B, is an indication that there is a considerable amount of guarana constituents still present in the plastic.

The results suggest that the method would be useful with even more dilute solutions, and quantitation is possible.

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Alkaloids of the *Papaveraceae* V. Muramine and Berberine from *Argemone squarrosa*

By FRANK R. STERMITZ*

***Argemone squarrosa* Greene (subsp. *squarrosa*) from several locations in central New Mexico was found to contain mainly muramine and berberine, but no allocryptopine. This is in contrast to an earlier literature finding that the same species from southern Colorado contained allocryptopine as the major, if not only, alkaloid. Muramine is now available for the first time in quantities sufficient for pharmacological testing.**

DIVISION of the genus *Argemone* into four tentative alliances based upon chemical and morphological criteria was recently suggested (1). Preliminary chemical studies (2) on *A. polyanthemos* (Fedde) Ownb., *A. corymbosa* Greene (subsp. *arenicola* Ownb.), *A. chisosensis* Ownb., and *A. sanguinea* Greene have indicated that each of these has berberine, protopine, and allocryptopine as major alkaloids. Chemically, these species are thus similar to *A. mexicana* L., *A. ochroleuca* Sweet, *A. aenea* Ownb., and *A. albiflora* Hornem, for all of which some data were already present in the literature. Two of the more tentative alliances were suggested (1) by dividing the above species into two groups based upon morphological criteria (3, 4). The two remaining alliances were based on the presence of pavine-type alkaloids either almost exclusively (*A. hispida* Gray and *A. munita* Dur. and Hilg.) or along with major concentrations of berberine and protopine-type alkaloids (*A. platyceras* L. and O.). Additional preliminary studies (5) have shown that *A. gracilenta* Greene is very similar in alkaloid content to *A. hispida* and that *A. pleiacantha* Greene and *A. platyceras* are closely related. Slavik and Slavikova had earlier suggested (6) somewhat similar alliances, although the number of investigated species was considerably smaller at that time.

Morphologically, *A. squarrosa* Greene was tentatively suggested (4) to be allied to *A. hispida*, but

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the only chemical investigation (7) reported that β-allocryptopine (I*a*) was essentially the only alkaloid present in *A. squarrosa* Greene (subsp. *squarrosa*) collected in southern Colorado. Thus, this species seemed to be one which could not be placed in any of the above alliances with certainty. In the course of a collecting trip in New Mexico it was noted that *A. squarrosa* in most locations had a bright yellow sap, which has been found indicative of considerable concentrations of berberine. It was therefore deemed advisable to chemically investigate the species from this area.

Collections were made at two locations in Lincoln County, where *A. squarrosa* (subsp. *squarrosa*) had previously been collected (4), and one location in Eddy County, where this subspecies had been reported (4) to grow mixed with *A. squarrosa* (subsp. *glabrata*). Each of the three collections was found to contain approximately 0.5% alkaloids, with the Lincoln County plants containing about a 4:1 ratio of muramine (I*b*) to berberine. This ratio was approximately reversed in the Eddy County plants. Only traces of other alkaloids could be detected by thin-layer chromatography of the last mother liquors remaining after removal of muramine and berberine. Because of the similarity of structure and melting point of muramine and β-allocryptopine, an authentic sample of the latter, which had been isolated (7) from the same plant species, was obtained[1] and compared with I*b*. The two were indeed found to be different and the original assignment of structure (7) of the β-allocryptopine was confirmed by additional spectral methods.

On the basis of these results and considering plant morphology, *A. squarrosa* is therefore tentatively assigned to the alliance composed of *A. polyanthemos*, *A. corymbosa*, *etc.* It still remains somewhat distinctive, however, in that all other members of the alliance contain protopine, allocryptopine, and berberine and apparently do not contain muramine.

Muramine has, up to now, only been isolated in trace amounts from *Argemone* (8) and a few *Papaver* species (9) and its synthesis (10) has only recently been accomplished in small yield. The discovery of a good source has now provided sufficient material for pharmacological testing, the results of which will be reported at a later date.

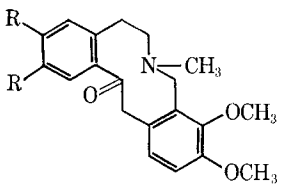
EXPERIMENTAL[2]

Material—The three collections of *A. squarrosa* (subsp. *squarrosa*) used in the present study were made in June 1966 as follows:[3]

(*a*) Lincoln County, N. Mex., 2 mi. east of Capitan on U.S. highway 380; in blossom, but capsules rare. Voucher sample: No. 111162.

(*b*) Lincoln County, N. Mex., east city limits of Lincoln; in blossom, but past peak blossom time. Many capsules. Voucher sample: No. 111156.

(*c*) Eddy County, N. Mex., 4.9 mi. west of Hope on U.S. highway 82; similar growth stage to collection (*b*). The presence of *A. squarrosa* (subsp. *glabrata*) as well as intermediates between the two subspecies was noted at this location, but the attempt was made to collect homogeneous subsp. *squarrosa*. Voucher sample: No. 111155.



I*a*, R, R = —O—CH_2—O—
I*b*, R′ = R = OCH_3

Isolation and Identification of Alkaloids—The following description [for that of collection (*a*) above] is typical. A total of 521 Gm. of dried, ground whole plant material was extracted for 24 hr. with methanol in a Soxhlet extractor. The methanol was evaporated to a viscous liquid and this was partitioned between 1 *M* sulfuric acid and a 1:1 *n*-butyl alcohol–benzene solution. Some insoluble tar was discarded. After the layers were separated, the organic layer was extracted with further portions of acid. These were combined and washed once with chloroform. The combined aqueous acid solution was made basic with sodium carbonate and extracted three times with equal volumes of chloroform. The chloroform solution was dried with anhydrous sodium sulfate and evaporated *in vacuo* to leave 2.4 Gm. of brown-yellow gum. This was dissolved in 30 ml. of hot methanol and cooled. Brownish transparent prisms (0.38 Gm., m.p. 168–172°) were formed and these were recrystallized once from methanol to yield pure transparent prisms of muramine, m.p. 174–176°. [Lit. (11) m.p. 176–177°.] This was spectroscopically identical to muramine isolated (8) from *A. munita* (subsp. *rotundata*). The methanolic solution from the first muramine crystallization was evaporated to dryness and dissolved in 30 ml. of warm, dilute hydrochloric acid. Cooling the solution yielded yellow-brown microcrystals (0.09 Gm.) of berberine chloride which were spectroscopically identical to a commercial sample. From these results, further work-up of the mother liquors and thin-layer chromatography of residues, the ratio of muramine to berberine in the total alkaloid fraction was estimated at 4:1. Only by heavily loading thin-layer plates with final residues could traces of two other alkaloids be observed. These were not further isolated. Since it was found (7) that ethanol Soxhlet extraction removed only a portion of the allocryptopine from Colorado *A. squarrosa*, the plant residue from the present methanol extraction was submitted to the standard (8) direct butanol–benzene method, but no further alkaloids were obtained.

Collection (*b*) yielded results not differing significantly from those just described. However, when the same procedure was applied to collection (*c*) the first crystallization of the total crude alkaloid fraction yielded berberine, not muramine. Only after redissolving residues in acid and repeating the cycle of extractions and pH adjustment could muramine be isolated. The final results from collection (*c*), again based on isolated amounts plus thin-layer chromatographic estimation of residue

[1] The author is indebted to Professor Soine for this sample.

[2] Melting points were determined on a Mel-Temp apparatus and are uncorrected. Spectral comparisons were made using an Hitachi Perkin-Elmer RMU6-E mass spectrometer and Varian A-60 (NMR), Beckman IR-8 (IR), and Cary 15 (UV) spectrometers. The purchase of the mass spectrometer was made possible through a grant from the National Science Foundation to the Department of Chemistry.

[3] The author is indebted to Professor G. B. Ownbey for confirming the identity of each of the above voucher samples. The numbers given are accession numbers in the Intermountain Herbarium, Utah State University.

contents, showed an approximate ratio of berberine to muramine of about 4:1. This reversal of concentrations might be related to environmental factors or to a genetic nonhomogeneity of the subspecies. (See under *Materials*.)

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Alkaloids of the *Papaveraceae* VI. Protopine and Allocryptopine from *Arctomecon californica*

By FRANK R. STERMITZ* and V. P. MURALIDHARAN

Protopine and allocryptopine (in approximately equal amounts) make up over 95 per cent of the alkaloidal content of *A. californica*.

THE GENUS *Arctomecon* consists of three species (*A. californica* Torr. et Fremont, *A. humilis* Coville, and *A. merriami* Coville) whose range is restricted to the extreme southwestern corner of Utah, southern Nevada, and the adjoining California desert (1). As part of the continuing investigation of the *Papaveraceae*, it was deemed advisable to investigate this relatively rare genus. Although extensive searches were conducted in 1962–1964 at locations where previous collections of each species had been made, only *A. californica* could be found.[1]

From 700 Gm. of powdered stems and leaves, a total of 2.3 Gm. of crude alkaloid mixture was obtained after treating the powder with 1:1 butanol–benzene, extracting this solution with 1 *M* sulfuric acid, adjusting to pH 9, extracting with chloroform, and evaporating the chloroform layer to dryness. Thin-layer chromatography showed the presence of two alkaloids. A small portion of root material treated in the same fashion showed similar results except with a slightly higher total alkaloid content.

The alkaloid mixture was treated with hot methanol, and protopine was precipitated from the cooled solution. Identification was made by NMR, U.V., and I.R. comparison with a known sample (2). Evaporation of the mother liquor, followed by crystallization of the residue from methanol, was accomplished three times with each crystallization yielding protopine. The fourth crystallization yielded a different alkaloid (m.p. 150°) which, after seven further recrystallizations, had m.p. 156° and was identical in all respects (NMR, I.R., U.V.) to α-allocryptopine, m.p. 160°.[2]

Thin-layer chromatographic analysis of the mother liquors again showed mainly protopine and allocryptopine. An additional two spots could be faintly seen, but sufficient material for further work was not available. Based on the isolated pure alkaloids and thin-layer chromatographic analysis of the mother liquors, protopine and allocryptopine occur in approximately equal amounts in *A. californica* and together represent probably 95% of the total alkaloid content.

General experimental procedures were as previously reported (3).

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[1] The authors are indebted to Nelson N. Williams, Department of Biology, University of Nevada (Southern), for this collection (made at Las Vegas, Nev.) and for his extensive attempts to locate other species of the genus *Arctomecon*.

[2] The authors are indebted to Dr. R. H. Manske for supplying the authentic sample.