(11) B. Starjecki, J. L. Reddin, and W. W. Spink, *Ann. Surg.*, **167**, 35(1968).
(12) G. J. Grega and W. J. Kinnard, *J. Pharmacol. Exp. Ther.*, **161**, 98(1968).
(13) G. J. Grega, W. J. Kinnard, and J. P. Buckley, *Circ. Res.*, **20**, 253(1967).
(14) J. A. Vick, H. P. Ciuchta, and J. H. Manthei, *J. Pharmacol. Exp. Ther.*, **150**, 382(1965).
(15) R. C. Hall and R. L. Hodge, *J. Physiol.*, **213**, 69(1971).

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Structure of Sanguidimerine, a New Major Alkaloid from *Sanguinaria canadensis* (Papaveraceae)

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Abstract □ Structure elucidation of a new major alkaloid, sanguidimerine, was based on the interpretation of its physical and chemical characteristics and was verified by partial degradation and semisynthesis. The possibility of the natural occurrence of sanguidimerine in *Sanguinaria canadensis* is also discussed.

Keyphrases □ *Sanguinaria canadensis* (Papaveraceae)—structure elucidation of sanguidimerine □ Sanguidimerine, alkaloid from *Sanguinaria canadensis*—structure □ Alkaloids—structure of sanguidimerine isolated from *Sanguinaria canadensis*

During a continuing search for anticancer compounds from plants, an investigation was initiated on the rhizomes of *Sanguinaria canadensis* because of its reputed use as a folk remedy and because certain alkaloids (*e.g.*, sanguinarine and chelerythrine) present in *S. canadensis* have been shown to possess antitumor activity (1). The study resulted in the isolation of sanguinarine and a new alkaloid designated as SC-2, which was assigned the trivial name sanguidimerine (2).

This report concerns the structure elucidation of sanguidimerine.

DISCUSSION

Sanguidimerine (I), a crystalline base, m.p. 174°, $[\alpha]_D^{26}$ +21.2° (concentration 0.5 in pyridine), was found to be only slightly soluble in most organic solvents and insoluble in water. It was determined earlier (2) that the UV absorption of this base (Table I) was very similar to that of the benzophenanthridine-type alkaloids and, particularly, to that of dihydrosanguinarine (3). A nonconjugated carbonyl was evidenced by a strong absorption at 1710 cm.$^{-1}$ in the IR spectrum.

Analysis by means of high resolution mass spectrometry (Tables II and III) showed that I exhibited a molecular peak at *m/e* 720, corresponding with a molecular formula of $C_{43}H_{32}N_2O_9$ (Table III). During the fragmentation (Scheme I), hydrogen transfer occurred, producing an ion at *m/e* 389 which was consistent as being identical to acetonyl dihydrosanguinarine, $C_{23}H_{19}NO_5$, and which further fragmented to give a base peak at *m/e* 332 This latter peak was verified by high resolution measurements (Table III) to be $C_{20}H_{14}NO_4$, *i.e.*, the sanguinarium ion (ion *b*). Formation of the sanguinarium ion was further evidenced by the presence of some diagnostic ions, *c*, *d*, and *e*, at *m/e* 317 (ion *b* − 15). 259 (ion *b* − 15 − 58), and 201 (ion *b* − 58 − 58 − 15), which were previously observed in the mass spectrum of sanguinarine pseudocyanide and related bases by Slavik *et al.* (4).

Treatment of I with hydriodic acid yielded sanguinarine as the iodide, and the stoichiometric ratio suggested that one part of sanguidimerine (I) produced two parts of sanguinarine iodide, which further established that the two parts of the dimer were identical (dihydrosanguinarine moiety) and were linked together by means of a C_3H_4O group which contained a carbonyl function.

Previously, a similar benzophenanthridine dimer alkaloid (5) was isolated from *Cheliodonium majus*. Upon comparison, it was found that these two alkaloids, sanguidimerine (I) and chelidimerine, provided identical IR, UV, and mass spectra. However, they differed with respect to melting points (174° *versus* 258–260°), solubility characteristics, and optical rotations. While sanguidimerine was only slightly soluble in most organic solvents, chelidimerine was readily soluble in these solvents. A positive specific rotation was observed for sanguidimerine, whereas chelidimerine was optically inactive. It was, therefore, logical to assume that these two bases were isomers, sanguidimerine being the optically active (+) compound. Preliminary single-crystal X-ray crystallographic data suggested that chelidimerine was a *meso*-compound (5). The data thus suggested sanguidimerine (I) to be (+)-1,3-bis(11-hydrosanguinarinyl)acetone.

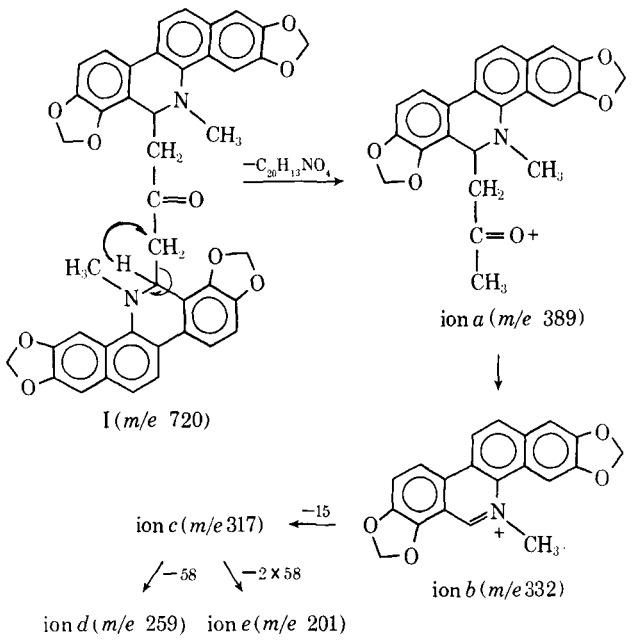
Table I—UV Spectra of Sanguidimerine and Dihydrosanguinarine

Alkaloid	λ_{max}, nm. (log ε)	Reference
Sanguidimerine	235 (4.65); 284 (4.63); 323 (4.23)	2
Dihydrosanguinarine	236 (4.62); 284 (4.62); 323 (4.28)	3

Table II—Mass Spectrometric Data for Sanguidimerine

m/e	Abundance, %[a]
720	4
389	2
362	2
332	100
317	12
316	2
274	4
259	3
201	3

[a] Spectra were obtained using a double-focusing mass spectrometer MS9, Allied Electrical Instrument Industries Ltd., Manchester, England.



Scheme I—Mass Spectral Fragmentation of Sanguidimerine

To confirm the structure, the synthesis of sanguidimerine was done according to the procedure of MacLean *et al.* (6) for the synthesis of bis(11-hydrochelerythrine)acetone. This was accomplished by the base-catalyzed condensation of sanguinarine and freshly prepared acetonedicarboxylic acid. The resulting product gave IR, UV, and mass spectra features identical with those of sanguidimerine.

Inasmuch as this common plant *S. canadensis* had been studied extensively, it seemed surprising that a major alkaloid such as sanguidimerine had eluded earlier workers. It is presumed that this type of alkaloid was missed previously because of the drastic nature of the isolation techniques employed by earlier investigators (7–9). For example, the scheme utilized by Slavík and Slavíkova (9) utilized pseudocyanide formation as an aid in the isolation of alkaloids from *S. canadensis*. The benzophenanthridine alkaloids present in *S. canadensis* are pseudobases. Hence, there exists a possibility of undesirable side reactions occurring due to the cyanide ion or other nucleophilic agents utilized during these isolation procedures. The success of isolating a new major alkaloid having a novel structure may, therefore, be attributed to the rapid and less drastic isolation methods employed in our work.

MacLean *et al.* (6) also recently reported the isolation of an alkaloid similar to sanguidimerine, and they ruled out the possibility of it being an artifact. A recent isolation of corynolamine from *Corydalis incisa* and bocconoline from *Macleaya cordata* by Ishii *et al.* (10) demonstrated the possibility of a biogenetic introduction of the carbon unit at C_{11} of the benzophenanthridine nucleus. This further supports the possibility of the natural occurrence of sanguidimerine in *S. canadensis*. In fact, it may not be presumptuous to speculate that these types of alkaloids are real natural products, and certain of the monomeric alkaloids reported previously may be at least partially artifacts produced as a result of the isolation procedures employed.

EXPERIMENTAL

Conversion of Sanguidimerine to Sanguinarine (Iodide)—Hydriodic acid (57%) was added dropwise to a methanolic solution containing 0.10 g. of sanguidimerine until the solution was acidic. The resulting solution, upon chilling, gave crystals. Recrystallization from acetone gave red needles (0.08 g.) melting at 229° dec. The product was identified as sanguinarine iodide [identical melting point, IR, UV, and mass spectra with an authentic sample (2)].

Synthesis of 1,3-Bis(11-hydrosanguinarinyl)acetone—Sanguinarine (100 mg.) was dissolved in pyridine (5 ml.), and freshly prepared acetonedicarboxylic acid (30 mg.) was added according to the method of MacLean *et al.* (6). Pyridine was removed *in vacuo*, and the residue was chromatographed over a column packed with silicic acid–diatomaceous earth[1] (4:1) in benzene; a blue UV fluorescent band was eluted with benzene–chloroform (9:1). This fraction was taken to dryness *in vacuo*, and the residue was crystallized several times from chloroform–methanol (3:1) to yield 20 mg. of colorless plates, m.p. 160–164° and 250–258°; $[\alpha]_D^{24} \pm 0°$ (concentration 0.5 in chloroform). The IR, UV, and mass spectra of this synthetic product were identical with that of the isolated chelidimerine.

Table III—High Resolution Measurements of Selected Ions for Sanguidimerine[a]

Ion Composition	Observed *m/e*	Calculated *m/e*
$C_{43}H_{32}N_2O_9$	720.2126	720.2108
$C_{23}H_{19}NO_5$	389.1261	389.1263
$C_{21}H_{16}NO_5$	362.1011	362.1028
$C_{20}H_{14}NO_4$	332.0929	332.0923

[a] Spectra were obtained using a double-focusing mass spectrometer MS9, Allied Electrical Industries Ltd., Manchester, England.

Biological Activity of Sanguidimerine—Sanguidimerine was evaluated for antitumor activity in the 5WA (Walker carcinosarcoma 256, subcutaneous) and 3PS (P-388 leukemia) test systems and for cytotoxicity in the 9-KB carcinoma of the nasopharynx in cell culture according to established protocols (11). The alkaloid was inactive in the WA and PS test systems, and gave ED_{50} of 2.7×10^1 mcg./ml. in the 9-KB assay. The minimal acceptable standards for cytotoxicity are $ED_{50} \leqq 1.0$ mcg./ml.

REFERENCES

(1) J. L. Hartwell, *Cancer Chemother. Rep.*, **7**, 19(1960).

(2) M. Tin-Wa, N. R. Farnsworth, H. H. S. Fong, and J. Trojánek, *Lloydia*, **33**, 267(1970).

(3) J. Holubek and O. Strouf, "Spectral Data and Physical Constants of Alkaloids," vol. I, Academia, Publishing House of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia, 1965.

(4) J. Slavik, L. Dolejs, V. Hanus, and A. D. Cross, *Collect. Czech. Chem. Commun.*, **33**, 1619(1968).

(5) M. Tin-Wa, H. K. Kim, H. H. S. Fong, N. R. Farnsworth, J. Trojánek, and D. J. Abraham, *Lloydia*, **35**, 87(1972).

(6) D. B. MacLean, D. E. F. Gracey, J. K. Saunders, R. Rodrigo, and R. H. F. Manske, *Can. J. Chem.*, **47**, 1951(1969).

(7) T. Kozniewski, *Bull. Int. Akad. Sci. Cracovie*, 235, **1910**(A); through *Chem. Abstr.*, **5**, 2103(1910).

(8) G. Schenck and H. Hanse, *Arch. Pharm.*, **277**, 355(1954); through *Chem. Abstr.*, **52**, 8460d(1954).

(9) J. Slavik and L. Slavikova, *Collect. Czech. Chem. Commun.*, **25**, 1667(1960).

(10) H. Ishii, K. Hosoya, and N. Takao, *Tetrahedron Lett.*, **1971**, 2429.

(11) Anon., *Cancer Chemother. Rep.*, **25**, 1(1962).

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