Biological and Phytochemical Evaluation of Plants V: Isolation of Two Cytotoxic Alkaloids from *Chelidonium majus*

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Abstract □ A routine screening of randomly selected native plants for biological activity revealed that an extract from *Chelidonium majus* elicited significant cytotoxicity, but was devoid of antitumor properties. A subsequent investigation of the alkaloids from this plant resulted in the isolation of coptisine as the chloride. Coptisine chloride and a second new alkaloid have been shown to be two of the cytotoxic principles of *Chelidonium majus*.

Keyphrases □ *Chelidonium majus*—alkaloids, isolation □ Coptisine, *C. majus*—isolated, identified □ Cytotoxicity—*C. majus* alkaloids □ TLC—separation, identity ■ UV spectrophotometry—identity □ IR spectrophotometry—identity □ Mass spectroscopy—identity

The authors have been engaged for some time in the collection, extraction, and biological evaluation of native plants which were collected at random (1, 2). *Chelidonium majus* L. (*Papaveraceae*) extracts were found to be devoid of antitumor properties against four different neoplasms, but they had some antibiotic activity against four test organisms, *in vitro*, and displayed minor activity against the herpes simplex virus in tissue culture experiments (2). Subsequently, extracts of *C. majus* were submitted to the Cancer Chemotherapy National Service Center for evaluation against additional tumors and for cytotoxicity testing. It was determined that these extracts were also free from antitumor properties, but induced a significant degree of cytotoxicity against Eagle's 9 KB carcinoma of the nasopharynx in cell culture. On the basis of these results, a phytochemical investigation was initiated. Since this plant is known to be rich in alkaloids, this group of phytoconstituents became the object of the authors' preliminary examination.

Prior to the authors' work on this species, several substances were known to be present in *C. majus*. Among these are chelidonic acid, chelidoniol, rutin, quercitrin, choline, methylamine, histamine, tyramine (3), and the alkaloids chelerythrine (3–5), sanguinarine (3–5), chelilutine (4), chelirubine (4, 5), protopine (5, 6), α-allocryptopine (5), α-homochelidonine (7), chelidamine (8), chelamidine (9), chelamine (9), chelidonine (3, 5, 6, 9, 10), corysamine (9), (−)-stylopine (5, 9), (±)-stylopine [(±)-tetrahydrocoptisine)] (3, 5, 6, 9), coptisine (3, 5, 11), sparteine (12), methoxychelidonine (13), berberine (3), oxychelidonine (14), and oxysanguinarine (15).

Chelidonium majus has a long history in Europe as being useful for the treatment of colonic polyposis, by enema, and locally to remove warts, papillomas, condylomas, and nodules of nursing mothers (16, 17). Pukhalskaya *et al.* (18) and Sokoloff (19) have shown that extracts from this plant are inhibitory for sarcoma 180 and Ehrlich mouse carcinoma. These tumors were not used in the present investigation, thus the active antitumor principles of this plant must be very tumor specific in view of negative results in this laboratory.

Alkaloids from *C. majus* have antifungal properties (20), and inhibit and deform several protozoa (21, 22). Chelerythrine and sanguinarine inhibit bacteriophage type 1_{017} and T_2, as well as *Escherichia coli* in low concentrations (23, 24).

In 1896, Botkin reported two cases of carcinoma which responded to treatment with *C. majus* extracts (25), and Ivanoff used chelidonine sulfate in the treatment of gastric cancer (26). Berezkina used an extract of this plant topically in patients with cancer of the breast and other organs (27), and Denisenko has conducted clinical trials with *C. majus* extracts for cancer (28, 29).

Sanguinarine and chelerythrine (30), present in *C. majus*, show antitumor activity in animals, but apparently these alkaloids are too toxic for human use. Chelidonine and protopine, on the other hand, are cytotoxic, but are devoid of antitumor properties (19). Reports could not be found which would indicate that other alkaloids of *C. majus* have been evaluated for cytotoxicity or antitumor activity.

EXPERIMENTAL

Plant Material—The plant material used in this investigation was the rhizomes and roots of *Chelidonium majus* L. (*Papaveraceae*), collected in western Pennsylvania during the summer of 1967. Voucher specimens were identified and authenticated[1]. These specimens are deposited in the Herbarium of Carnegie Museum.

Alkaloid Extraction and Fractionation—A 500-g. sample of air-dried, coarsely milled rhizomes and roots of *Chelidonium majus* was percolated with a total of 30 l. of 5% acetic acid in methanol–water (1:1). The methanol was subsequently removed from the extract by means of a flash evaporator *in vacuo* at 40°. Five percent hydrochloric acid was added to the resulting aqueous acidic extract until a pH of 3.0 was reached. This acid solution was then extracted with a total of 8 l. of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, filtered, and taken to dryness *in vacuo* to yield 12.5 g. of alkaloid Fraction A.

The aqueous acidic solution remaining after removal of alkaloid Fraction A was then made alkaline to litmus paper with NH_4OH, followed by four 2-l. extractions with chloroform. Removal of the chloroform from the combined extracts *in vacuo* yielded 12.0 g. of Fraction B alkaloids.

Following these procedures, the partially extracted marc was air-dried and chloroform was added in sufficient quantity to just cover the drug. To this was added 300 ml. of 28% NH_4OH with concomitant stirring, which was sufficient to render the

[1] By Dr. LeRoy K. Henry, Carnegie Museum, Pittsburgh, Pa.

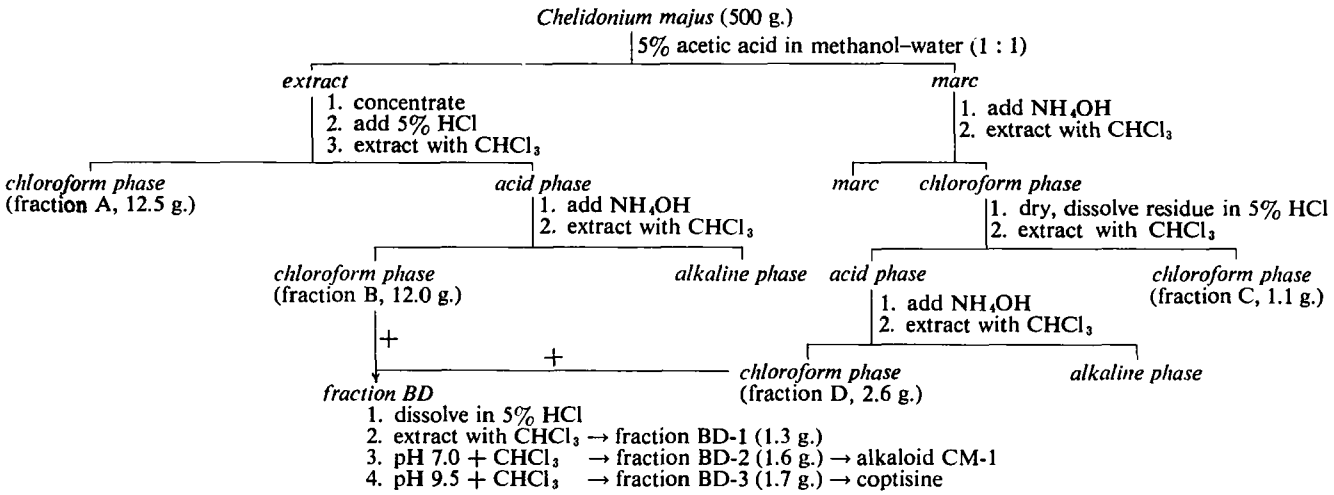


Figure 1—*Flow diagram for the extraction and fractionation of* Chelidonium majus *alkaloids.*

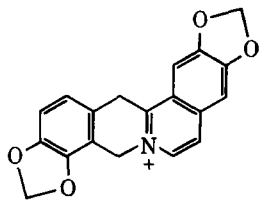
drug alkaline. This mixture was then percolated at a slow rate until 10 l. of extract was obtained. This chloroform extract was concentrated *in vacuo* to dryness and constituted the combined alkaloid Fraction C-D.

This combined C-D fraction was mixed with 500 ml. of 5% HCl, followed by stirring and application of gentle heat, followed by filtration. The filtrate was extracted with two 1-l. volumes of chloroform and the combined chloroform extracts were dried with sodium sulfate, and taken to dryness to yield 1.1 g. of alkaloid Fraction C. To the aqueous acidic solution remaining after the removal of the C fraction was added NH_4OH in sufficient quantity to render the solution alkaline to litmus paper. This was followed by two 1-l. extractions with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate, and taken to dryness *in vacuo* to yield 2.6 g. of alkaloid Fraction D.

TLC on silica gel G plates (250 μ), using solvent systems composed of either *n*-propanol–acetic acid–water (4:1:1), cyclohexane–diethylamine (9:1), or methanol–carbon tetrachloride–acetic acid (28:12:1), followed by spraying the formed chromatograms with modified Dragendorff's reagent (31), revealed that Fractions B and D contained the same major alkaloids. On this basis, they were combined to give a fraction which was designated as BD (14.6 g.).

Fraction BD was dissolved in 500 ml. of 5% HCl, filtered, and the filtrate extracted with three 500-ml. volumes of chloroform, and the combined chloroform extracts were taken to dryness *in vacuo* to give 1.3 g. of Fraction BD-1. The acidic aqueous phase was then adjusted to pH 7.0 with 14% NH_4OH followed by three 500-ml. extractions with chloroform, which after taking to dryness *in vacuo* yielded 1.6 g. of Fraction BD-2. Following removal of the BD-2 fraction, the pH of the aqueous phase was adjusted to 9.5 with 14% NH_4OH, and this solution was extracted with three 500-ml. volumes of chloroform. Removal of the chloroform from these combined fractions gave 1.7 g. of Fraction BD-3. A flow diagram of this extraction scheme is presented in Fig. 1.

Isolation of Coptisine Chloride—The alkaloid BD-3 fraction (1.7 g.) was dissolved in 50 ml. of 5% HCl, and water was removed *in vacuo* by means of azeotrope formation through the frequent addition of methanol. The resulting crude alkaloid chloride mixture was dissolved in a minimum volume of hot methanol, filtered, and chilled for 48 hr. During this period, yellowish-orange crystals deposited. Repeated crystallization from methanol, followed by drying at 100° *in vacuo* for 24 hr., gave 0.09 g. of needles having m.p. 266–268° dec. (cap., uncorr.), $[\alpha]_D^{25}$ 0° (c, 0.5, MeOH), and exhibiting an UV spectrum with absorption maxima at $\lambda_{max.}^{MeOH}$ 226 mμ (log ϵ 4.39), 239 mμ (4.35), 264 mμ (4.29), and 356 mμ (4.30). An IR spectrum (KBr) was also prepared. Comparison of these data with those derived from analysis of a reference sample of coptisine chloride[2] established that the two alkaloids were indeed identical. The calculated molecular ion of 320 was confirmed by mass spectrometry.



Coptisine

Isolation of Alkaloid CM-1—The alkaloid BD-2 fraction (1.6 g.) showed one major spot following TLC on silica gel G plates (250 μ), using a solvent system of *n*-propanol–acetic acid–water (4:1:1) (R_f 0.80) or cyclohexane–diethylamine (9:1) (R_f 0.13). This alkaloid fraction was dissolved in a minimum volume of hot chloroform–methanol (3:1), the solution filtered, and then chilled for 24 hr. During this period rhomboid crystals formed, which were subsequently removed by filtration, and recrystallized under the same conditions. Drying *in vacuo* at 100° for 24 hr. gave 0.050 g. of crystalline alkaloid having m.p. 258–260° (cap., corr.), and which exhibited an UV absorption spectrum with absorption maxima at $\lambda_{max.}^{MeOH}$ 232 mμ (log ϵ 4.59), 285 mμ (4.43), and 324 mμ (3.89), which is indicative of the benzophenanthridine nucleus. An IR spectrum was obtained (KBr) which exhibited the following characteristic absorption maxima: $\nu_{max.}$ 3.42 μ(m), 5.84 μ(s), 6.21 μ(w), 6.95 μ(s), 7.35 μ(m), 8.00 μ(s), 9.61 μ(s), 10.60 μ(s), 11.60 μ(s), 12.5 μ(m), and 13.30 μ(s).

Analysis by means of high-resolution mass spectrometry showed that the alkaloid has a molecular formula of $C_{23}H_{19}NO_5$ (calcd., 389.1263; obs., 389.1272).

These data are not consistent with those of any known benzophenanthridine alkaloid from the genus *Chelidonium*, and it has been designated Alkaloid CM-1. Studies are now in progress to elucidate the structure of Alkaloid CM-1.

Biological Evaluations—An extract for testing was prepared from the rhizomes and roots of *Chelidonium majus*. The milled sample was percolated with a mixture of ethanol–water (1:1), followed by removal of the ethanol *in vacuo*, and freeze-drying of the aqueous portion. This resulted in an extract that was used for the biological evaluations.

Tumor testing was against the L-1210 leukemia in mice and against the Walker 256 carcinosarcoma in rats (32). In both cases, the extract was devoid of activity. Cytotoxicity was determined against Eagle's 9 KB carcinoma of the nasopharynx in cell culture (32), and this extract of *C. majus* exhibited an ED_{50} of 9.8×10^0, 6.4×10^0, 2.1×10^1, and 1.1×10^{-1} mcg./ml. in replicate tests.[3]

[2] The reference sample of coptisine chloride was generously provided by Dr. J. Slavik, Institute for Medicinal Chemistry, Purkyne University, Prague, Czechoslovakia.

[3] An $ED_{50} \leq 15$ mcg./ml. is considered as active for plant extracts (32).

Coptisine chloride (NSC-119754) exhibited ED_{50} 8.2×10^{-1} mcg./ml. and Alkaloid CM-1 exhibited $ED_{50} < 1.5 \times 10^{0}$ mcg./ml. against Eagle's 9 KB carcinoma.[4]

SUMMARY

An investigation of *Chelidonium majus* L. (*Papaveraceae*) rhizomes and roots has shown that they are devoid of activity against the L-1210 leukemia and the Walker 256 (intramuscular) carcinosarcoma. However, an identical extract displayed significant cytotoxicity against Eagle's 9 KB carcinoma of the nasopharynx in cell culture.

The alkaloids of the rhizomes and roots of *C. majus* were examined and coptisine was isolated as the chloride, in addition to a second alkaloid designated as CM-1. These alkaloids were shown to be two of the cytotoxic principles of *Chelidonium majus*.

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[4] An $ED_{50} \leq 1.0$ mcg./ml. is considered as active for pure compounds (32).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 19, 1968, from the *Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213*

Accepted for publication December 12, 1968.

This investigation was supported in part by research grants CA-10850 and FR-05455 from the National Institutes of Health, U. S. Department of Health, Education and Welfare, Bethesda, MD 20014, and from the Jane Olmstead Thaw Fund, University of Pittsburgh.

Previous paper in this series, see A. B. Segelman and N. R. Farnsworth, *Lloydia*, in press.

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Daily Susceptibility Rhythm to Morphine Analgesia

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Abstract □ Mice exhibit a daily susceptibility to morphine-induced analgesia, that is, maximum sensitivity (crest) at 2100–2400 hr. in the dark phase and a minimum (trough) at 1500 (light phase). Shifts in the crest and/or trough of from 3 to 6 hr. were evident on three experimental dates; however, the crests and troughs always remained within their respective light phases. A discussion is presented on the interrelationships among the rhythms for catecholamine metabolism, motor activity, and susceptibility to stimulant and depressant drugs, particularly morphine.

Keyphrases □ Morphine analgesia—daily response rhythm □ Susceptibility patterns, mice—morphine analgesia □ Light, dark phases—morphine analgesia □ Lunar phases—morphine analgesia

Several drugs have been demonstrated to possess persistent daily or circadian (about 24-hr.) susceptibility patterns in rodents standardized in an alternating light-dark regimen (1–11). The resultant susceptibility pattern of these drugs when tested at frequent intervals is usually characterized by the temporal placement within a 24-hr. period of a major peak (crest) followed by a minimum (trough) approximately 12 hr. later. The times for the crest and trough responses to a specific drug can be reproduced *reliably* providing all studies are performed under identical conditions. Until a recent preliminary report on morphine from this laboratory (11), no response pattern had been shown for an analgesic agent. The present study was designed to