Alkaloids of *Scopolia carniolica*

By SANTO W. ZITO and JOHN D. LEARY

The alkaloid composition of the rhizomes of *Scopolia carniolica* Jacq. has been investigated and the identification of six alkaloids is reported. Three of these alkaloids have been reported previously and are confirmed in this report. Moreover, the authors report here for the first time the presence of 3-α-tigloyloxytropane in the genus *Scopolia;* in addition, pseudotropine and cuscohygrine are reported in this plant for the first time. One other alkaloid could not be identified at this time.

SCOPOLIA CARNIOLICA Jacq., a member of the family *Solanaceae*, is well known for its content of the tropane ester alkaloids hyoscyamine and scopolamine, and for the hydramine, tropine (1). Evidence for the existence of alkaloids other than those mentioned is without firm foundation. Bendik *et al.* (2) detected the presence of scopine in addition to the previously mentioned bases, and Schreiber (3) reported on the presence of solanidine in this plant as well as in many other members of the nightshade family; neither of these bases could be confirmed in this work. Another report suggests the presence of alkaloids which were not identified (4).

The dried rhizomes were extracted by two different methods, subjected to preliminary purification, and were analyzed for the presence of alkaloids by paper partition and thin-layer chromatographic procedures. Examination of the chromatograms revealed the presence of seven alkaloids, three of which were previously identified.

EXPERIMENTAL

Extraction.—The dried rhizomes[1] were extracted by two different procedures. The first method involved continuous extraction with methanol in a Soxhlet apparatus (5) until devoid of alkaloids when tested with Mayer's reagent. The methanol extract was concentrated to a viscous solution at 40° *in vacuo*, diluted with water, and acidified to pH 2 with hydrochloric acid. The aqueous solution was exhaustively extracted with chloroform (fraction A). The aqueous phase was then adjusted to pH 10 with 20% sodium hydroxide solution and extracted anew with chloroform (fraction B). Each of these fractions was submitted to analysis by paper and thin-layer chromatography.

In the second method the drug was moistened overnight with water and was then intimately mixed with calcium hydroxide according to the method of Evans and Stevenson (6). The basified drug was stirred with solvent ether during the next 2 hr.; the supernatant liquid was decanted, and the marc was further percolated with solvent ether until the percolate gave a negative test for the presence of alkaloids. The ether extracts were combined and concentrated at 40° *in vacuo;* the viscous extract was fractionated on a buffered kieselguhr column.

Column Partition Chromatography.—The concentrated extracts from Evans' method were placed on a column of kieselguhr (30 Gm.) loaded with phosphate buffer (15 ml.; pH 6.6). Elution was carried out with successive portions of petroleum ether, solvent ether, chloroform, and chloroform saturated with concentrated ammonium hydroxide solution. Petroleum ether gave an oily material which gave a questionable Dragendorff-positive spot of high R_f value on paper and thin-layer chromatograms (see below). Ether eluted hyoscine followed by 3-α-tigloyloxytropane; chloroform removed hyoscyamine and an unidentified material. The ammoniacal chloroform afforded tropine, pseudotropine, and cuscohygrine. Identifications were based on elution patterns on the buffered column which coincided with previously reported results (6), as well as by comparison of the extracts with known compounds in the chromatographic systems mentioned later.

Paper Partition Chromatography.—Whatman No. 1 paper was impregnated with 0.5 *M* KCl solution, air dried, and used for the separation of the various extracts and reference compounds,[2] used singly and in mixtures. Development of the chromatograms was achieved with the solvent system of Rother *et al.* (7) which consisted of *n*-butanol–concentrated hydrochloric acid (98:2) saturated with water. Revelation of the alkaloids was effected with modified Dragendorff's reagent (8). Two other partition systems were used but were not as successful in complete resolution of the mixtures.

Thin-Layer Chromatography.—A matrix of aluminum oxide G (Brinkmann) was prepared by the standard procedure (0.250 mm.), and the plates were activated at 110° for 30 min. Extracts of the plant in addition to reference compounds, singly and admixtures, were developed with a solvent system of benzene–methanol (9:1); extracts suspected of containing cuscohygrine were developed with a mixture of benzene–methanol–diethylamine (99:1:5). Revelation of the alkaloids was achieved with modified Dragendorff's reagent.

The data for the chromatographic studies are summarized in Table I.

RESULTS

Extracts of the rhizomes of *S. carniolica* obtained by two different methods have yielded fractions which were analyzed by several paper and thin-layer chromatographic procedures. Maximum resolution was achieved in the two systems which are reported. The development of purple spots in the case of tropine and pseudotropine-containing extracts aided materially in their identification. Identification was effected by means of elution patterns on a kieselguhr-phosphate buffer column,

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[1] The *S. carniolica* Jacq. rhizomes used in this study were generously supplied by S. B. Penick Co., New York, N. Y.

[2] Hyoscyamine and scopolamine (Merck) were obtained commercially; cuscohygrine, tropine, pseudotropine, and 3-α-tigloyloxytropane were supplied by Dr. A. E. Schwarting University of Connecticut, Storrs; a second sample of the latter was supplied by Dr. W. Evans, University of Nottingham, Nottingham, England.

TABLE I.—CHROMATOGRAPHIC DATA FOR THE ALKALOIDS OF *S. carniolica*

Alkaloid	Paper[a]	Thin-Layer[b]
Cuscohygrine	0.08	0.00; 0.63[c]
Pseudotropine	0.15	0.13
Scopolamine	0.26	0.52
Unidentified alkaloid	0.31	0.63
Tropine	0.34	0.22
Hyoscyamine	0.48	0.43
3-α-Tigloyloxytropane	0.84	0.63

[a] Whatman No. 1 paper (0.5 *M* KCl); *n*-butanol–HCl (98:2) water-saturated. [b] Aluminum oxide G; benzene–methanol (9:1). [c] Aluminum oxide G; benzene–methanol–diethylamine (99:1:5).

comparison of R_f values of compounds in extracts when analyzed by paper and thin-layer chromatographic procedures with authentic compounds, and color reactions.

The data indicate the presence of at least seven alkaloids. Identification of hyoscyamine, scopolamine, and tropine corroborates the studies of previous workers. This work has succeeded in the tentative identification of 3-α-tigloyloxytropane, the first reported occurrence of this compound in the genus *Scopolia;* it extends the distribution to still another genus of the family *Solanaceae* along with *Datura* (6), *Withania* (5), and *Physalis* (9). It also broadens the knowledge of the existence of cuscohygrine in plants containing tropane alkaloids. Pseudotropine was the sixth alkaloid to be identified. One other alkaloid remains to be identified. Failure to confirm the presence of scopine and solanidine in this sample was due to a lack of authentic reference compounds.

Work is continuing and complete details on the extraction, isolation, and characterization will be published at a later date.

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Synthesis of *N,N'*-Haloacyl Analogs of *p,p'*-Oxydianiline as Potential Antineoplastic Agents

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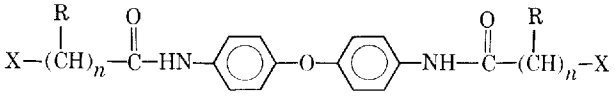
A series of eight new haloacetyl and halopropionyl derivatives of *p,p'*-oxydianiline have been synthesized for evaluation of anticarcinogenic activity.

AS AN INTEGRAL part of this continuing cancer chemotherapy research project, another series of bis-haloamide analogs of a parent diamine molecule have been prepared. Based upon the screening data obtained in this laboratory (1–3) and that from others in the field (4–7), the chemotherapeutic activity of such compounds is deserving of further study. This report concerns itself with the synthesis of a series of bis-haloacetyl and bis-halopropionyl derivatives of *p,p'*-oxydianiline (I).

This type of alkylating agent may inhibit the growth of cancer cells through selective inhibition of vital metabolic activities within tumor cells (8–15). By varying the carrier moieties of these active, relatively nontoxic compounds, it is hoped that some insight will be gained as to structure–activity relationships as regards their alkylating abilities.

DISCUSSION

An anhydrous chloroform solution of the diamine, *p,p'*-oxydianiline[1] (I), was treated with a chloroform solution of chloroacetyl chloride, 2-chloropropionyl chloride, and 3-chloropropionyl chloride to form,



X = Br, Cl, and I
R = H, n = 1,2
R = CH_3, n = 1

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[1] Supplied by The Dow Chemical Co., Midland, Mich.