Isolation of Two Bisbenzylisoquinoline Alkaloids from the Rhizomes and Roots of *Xanthorhiza simplicissima*

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The alkaloids obamegine and oxyacanthine were isolated from the tertiary alkaloid fraction of the rhizomes and roots of *Xanthorhiza simplicissima*. The tertiary alkaloids were separated from the quaternary fraction using a chromatographic column of a polyamide (nylon 66). The mixture of obamegine and oxyacanthine was separated by partition chromatography.

DURING THE course of the investigation of the quaternary alkaloids of the rhizomes and roots of *Xanthorhiza simplicissima* Marsh., a quantity of tertiary alkaloid material was isolated (1). This investigation was undertaken to isolate and identify the alkaloids in the tertiary fraction.

EXPERIMENTAL[1]

Plant Material.—The rhizomes and roots of *X. simplicissima* Marsh. used in this investigation were obtained in granulated form from S. B. Penick and Co. (lot B-19753).

Extraction.—Twenty kilograms of the plant material was extracted with ethanol U.S.P. by percolation at room temperature. Extraction was continued until the extractive failed to give a positive test with Valser's solution (2). Evaporation of the ethanol *in vacuo* at 40° or less yielded 1.7 Kg. of residue which was dissolved in 5% hydrochloric acid solution and filtered. The filtrate was basified to pH 8–9 (indicated by pHydrion paper) with 10% ammonium hydroxide solution and extracted with ethylene dichloride. After drying over anhydrous sodium sulfate the ethylene dichloride was removed *in vacuo* to yield 19.6 Gm. of crude tertiary alkaloids. Thin-layer chromatography on Silica Gel G,[2] using a solvent system of benzene–acetone–ammonium hydroxide (32:32:1) indicated the presence of only two tertiary alkaloids in the mixture; a spot at the origin was also evident indicating the presence of quaternary alkaloids in the mixture. The alkaloid spots were detected with Dragendorff's spray reagent (3). By means of a modification of a spot test for phenols (4) it was possible to determine on the thin-layer chromatogram that both tertiary alkaloids were phenolic. This was performed by spraying the developed chromatogram with a 2% solution of phosphomolybdic acid in 50% aqueous acetone and then exposing the chromatogram to ammonia fumes. Phenolic compounds will give a blue to black color with this treatment.

Separation of the Quaternary from the Tertiary Alkaloids.—The small quantity of quaternary alkaloids present in the crude tertiary alkaloid fraction were removed by utilizing a chromatographic column prepared with a polyamide (nylon 66) powder.[3] Two glass chromatographic columns (4 × 40 cm.) each containing 200 Gm. of the polyamide powder were prepared. One column was prepared by the slurry method in water while the other by the slurry method in benzene. The crude alkaloid mixture (19.6 Gm.) was dissolved in chloroform and adsorbed onto about 5 Gm. of the polyamide powder followed by evaporation of the solvent. The powder was placed on the column prepared with water and the column eluted with water. Several colored, fluorescent bands were eluted and were shown by thin-layer chromatography to be traces of quaternary alkaloids. The column was next eluted with methanol and the effluent reduced in volume and again adsorbed onto about 5 Gm. of the polyamide powder as before. The resulting powder was placed on the column prepared with benzene and the column eluted with benzene until the eluate was alkaloid free (as indicated by Valser's test). Evaporation of the benzene from the effluent, *in vacuo*, yielded 12.3 Gm. of semicrystalline material. This material was shown by thin-layer chromatography to consist mainly of the two phenolic alkaloids.

Separation of the Tertiary Alkaloids by Partition Chromatography.—The two tertiary alkaloids were purified by partition chromatography according to the procedure of Brown and Kupchan (5). The solvent system consisted of methanol–water (5:1, v/v) as the stationary phase and ethylene dichloride–Skellysolve F (1:2, v/v) as the mobile phase with a diatomaceous earth[4] serving as the inert support. Bromothymol blue was incorporated into the stationary phase to detect the alkaloid bands as they moved through the column. A chromatographic column (2 × 90 cm.) was prepared as follows. The solvent mixture of Skellysolve F, 1600

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[1] Melting points were determined with a Thomas-Hoover Uni-Melt capillary melting point apparatus. The infrared spectra were taken in chloroform and as KBr pellets on a Perkin-Elmer model 237 infrared spectrophotometer. The ultraviolet spectra were determined on a Perkin-Elmer model 4000 Spectracord.

[2] Made by E. Merck (Darmstadt, West Germany); obtained through Brinkmann Instruments, Inc., Cantiague Road, Westbury, N. Y.

[3] Obtained as Polypenco Powder from the Polymer Corp., Pittsburgh, Pa.

[4] Marketed as Hyflo Super-Cel by Johns-Manville Corp., New York, N. Y. Purified by suspending in 4 *N* HCl, with occasional agitation, for 24 hr. It was then filtered on a Büchner funnel and washed successively with distilled water, methanol, chloroform, benzene, and finally methanol again. After air-drying it was dried in an oven at 105° and stored in an air-tight container. Skellysolve F is a petroleum ether fraction, b.p. 30–60°. Ethylene dichloride C.P., Union Carbide Co., methanol A.R.

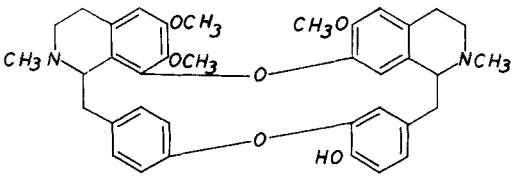
ml.; ethylene dichloride, 800 ml.; methanol, 300 ml.; and distilled water, 60 ml., was shaken well and allowed to equilibrate overnight. Two hundred grams of diatomaceous earth in a 1-L. glass-stoppered flask was treated with 132 ml. of the stationary phase (lower phase) of the solvent mixture, in which 60 mg. of bromothymol blue was dissolved. The flask was vigorously shaken to remove all lumps and the mixture then packed into the column in about 1-cm. increments. The mobile phase was then passed through the column until all the air had been displaced and the column was a uniform yellow color. A solution of 1.0 Gm. of the alkaloid mixture in 25 ml. of the mobile phase (upper phase) was added to the column. When the last portion of the alkaloid mixture had passed into the top of the column, elution was begun at a rate of about 100 ml./hr. The alkaloids were observed as two blue bands advancing down the column and were collected separately. The first band was labeled "alkaloid I" and the second "alkaloid II." Both bands were shown by thin-layer chromatography to consist of only one alkaloid. In this fashion, the remainder of the alkaloid mixture was separated.

Identification of Alkaloid I as Oxyacanthine.—The total effluent fractions containing alkaloid I were evaporated *in vacuo*. The residue was dissolved in ether and washed several times with a 10% sodium carbonate solution followed by distilled water. The ether solution was dried over anhydrous sodium sulfate followed by evaporation *in vacuo* to yield a residue of 4.75 Gm. which resisted all attempts of crystallization of the free base. The alkaloid was crystallized as the hydrochloride salt from a 5% hydrochloric acid solution. It was recrystallized from water, after saturation with sodium chloride, to give needles melting above 280° dec.

An aqueous solution of 1.4 Gm. of the hydrochloride salt was made basic with ammonia and extracted with chloroform. After drying over anhydrous sodium sulfate and removal of the chloroform *in vacuo*, a residue was obtained which crystallized from petroleum ether (b.p. 30–60°) as rosettes, m.p. 212–214°. $[\alpha]_D^{29}$ + 285.6° (c = 0.5, $CHCl_3$). The ultraviolet absorption spectrum was indicative of a benzylisoquinoline nucleus, having a $\lambda_{max.}^{MeOH}$ 283 mμ (log ε 3.95) (6). The infrared absorption spectrum in chloroform indicated a hydroxyl function ($\nu_{max.}$ = 3510 cm.$^{-1}$) in the molecule. The NMR spectrum in deuterochloroform gave indications of two *N*-methyl groups (7.40 and 7.45 τ, 3H each) and three *O*-methyl groups (6.25, 6.40, and 6.85 τ, 3H each) which suggested an oxyacanthine-type alkaloid with methoxyl groups at 6, 6′, and 7 positions, respectively, of the bisbenzylisoquinoline nucleus (7). The compound was identified to be oxyacanthine (I) on the basis of melting point, optical rotation, NMR data, and comparison of ultraviolet and infrared absorption spectra with an authentic sample of oxyacanthine.[5]

Identification of Alkaloid II as Obamegine.—The total effluent fractions containing alkaloid II were combined and treated in the same manner as for alkaloid I to yield 2.1 Gm. of residue. The material crystallized readily from benzene as colorless needles, m.p. 163–168° dec. $[\alpha]_D^{29}$ + 99° (c = 0.5, $CHCl_3$). The infrared spectrum in chloroform indicated the presence of hydroxyl function ($\nu_{max.}$ = 3525 cm.$^{-1}$). The ultraviolet spectrum gave $\lambda_{max.}^{MeOH}$ 280 mμ (log ε, 3.19).

The NMR spectrum[6] in deuterochloroform showed two *N*-methyl groups (7.6 and 7.75 τ, 3H each) and two *O*-methyl groups (6.3 and 6.4 τ, 3H each) which suggested a berbamine-type alkaloid with methoxyl groups at the 6 and 6′ positions of the molecule (7). The alkaloid was identified as obamegine (II) on the basis of melting point, optical rotation, NMR data, and comparison of infrared and ultraviolet absorption spectra with an authentic sample of obamegine.



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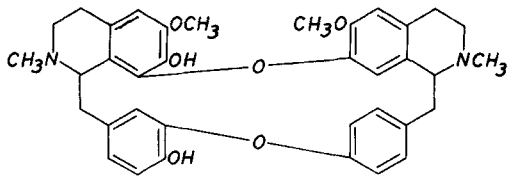

II

DISCUSSION

X. simplicissima is an example of a plant which when investigated with the advantage of more modern techniques such as chromatography was found to contain several more alkaloids than reported in the older literature. In 1862, Perrins (8) reported the isolation of berberine from the rhizomes and roots of the plant; Jones (9), in 1886 verified the presence of berberine and suggested the presence of a second alkaloid. Two additional quaternary alkaloids, jatrorrhizine and magnoflorine (1), were isolated in 1963. This investigation has shown that the rhizomes and roots contain the tertiary bisbenzylisoquinoline alkaloids oxyacanthine (1) and obamegine (II), thus bringing the total to five alkaloids reported to be isolated from *X. simplicissima* Marsh.

Oxyacanthine, first obtained in crystalline form by Spath and Kolbe in 1925 (10), has been found previously in eight species of the genus *Berberis* (*Berberidaceae*), seven species of the genus *Mahonia* (*Berberidaceae*), and in the species *Cocculus leaeba* (*Menispermaceae*) (11).

Obamegine was first isolated by Tomita and Kugo (12) in 1959 from *Berberis tschonoskyana* and recently by Tominatsu and Beal (13) from *Thalictrum rugosum* (*Ranunculaceae*). This is the third report of the isolation of this compound.

[5] The authors thank Dr. E. Fujita, Institute for Chemical Research, Kyoto University, Takatsuki, Osaku-fu, Japan, for an authentic sample of oxyacanthine.

[6] The authors thank Dr. David R. Dalton for determining the NMR spectra of obamegine and oxyacanthine. These were determined on a Varian A-60 NMR spectrometer.

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Ion-Exchange Separation and Ultraviolet Determination of Phenylephrine, Codeine, and Selected Antihistamines

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A method for the isolation and determination of phenylephrine, codeine, or an antihistamine as a single active ingredient is presented. Also, a method is presented for the separation and determination of an antihistamine and codeine or an antihistamine and phenylephrine in pharmaceutical products containing these combinations. All of the amines are extracted with a strong cation-exchange resin, AG 50W-X4. The phenylephrine or codeine is eluted with 1 *N* hydrochloric acid in 60 per cent methanol in water. The antihistamine is then eluted with 3.5 *N* hydrochloric acid in 40 per cent methanol in water. The compounds are determined by subjecting the eluates to ultraviolet spectrophotometry. The assay is used successfully on several commercial products.

A LARGE NUMBER of pharmaceuticals are presently marketed for treatment of the common cold and other respiratory disorders. These products use a large variety of active ingredients but for the most part they are, chemically, basic amines. These amines are in the form of vasoconstrictors, antitussives, and antihistamines.

Phenylephrine is widely used as a vasoconstrictor in these products. The assay for this compound in liquid pharmaceuticals has been difficult because of the water solubility of phenylephrine even as the base. The U.S.P. (1) describes the time honored bromination procedure. This procedure is ineffective when oxidizable substances are present because of their reactions with bromine. Reducing sugars are a good example of this type of interference. The reactions of phenols with ferricyanide and 4-aminoantipyrine has been proposed by several workers (2, 3). Chafetz (4) oxidized phenylephrine and other phenylethanolamines to their aromatic aldehydes, extracted with organic solvents, and determined the ultraviolet absorption. Clark and Rosenberg (5) reported phenylephrine separation using a Levine column with acetylation on the column. The phenylephrine was determined in the eluates. Auerbach (6) used a diazotization technique where phenylephrine acted as the coupler. Kelly and Auerbach (7) utilized ion-exchange chromatography in a method similar to that presented in this paper.

Codeine, which is a very popular antitussive, has been the subject of extensive analytical study for many years because of its wide usage. Nonaqueous titrimetry has been utilized by several workers (8–10). Artamonov and Bugreeva (11) applied the techniques of high frequency titration for the determination of codeine.

Several colorimetric methods have been proposed (12, 13) as well as fluorimetry (14, 15), refractometry (16), and spectrophotometry (17, 18).

Various types of chromatographic procedures have been used for codeine analysis. Izmailov and co-workers (19) used paper chromatographic techniques to separate codeine from other poppy extract alkaloids. Adsorption columns of silicic acid were utilized by Andreeva and Figurovskii (20). The more recent techniques of gas chromatography (21) and thin-layer chromatography (22) have also been reported.

The common methods of assay for antihistamines include nonaqueous titrimetry (23, 24), ultraviolet spectrophotometry (25), and reineckate salt formation with subsequent colorimetric determination of the acetone solution (26). A widely used colorimetric method, specific for compounds containing the 2-pyridyl group, is based on the König reaction with cyanogen bromide and aniline. This was described by Jones and Brody (27) and later modified by Hudanick (28). Gas chromatography has been used successfully by Celeste and Turczan (29) and by Fontan and co-workers (30).

This paper utilizes strongly acidic cation-exchange resins to separate the amines from common dosage form ingredients. The amines are then eluted separately from the resin using specific concentrations of hydrochloric acid. The amines are deter-

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