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Sinoacutine from *Glaucium contortuplicatum* Boiss.

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Abstract □ A phytochemical investigation of *Glaucium contortuplicatum* Boiss. (Papaveraceae) resulted in the isolation of sinoacutine from this plant for the first time. Spectral evidence for the identity of the isolated compound as sinoacutine is presented.

Keyphrases □ *Glaucium contortuplicatum*—phytochemical investigation, whole plant extract, sinoacutine isolated □ Sinoacutine—isolated from whole plant extract of *Glaucium contortuplicatum* □ Alkaloids—sinoacutine isolated from whole plant extract of *Glaucium contortuplicatum*

A literature survey revealed that no chemical work had been reported on the title plant. A phytochemical investigation was initiated, and the isolation of dicentrine nitrate was reported (1).

The isolation and identification of sinoacutine from this plant are now reported. Sinoacutine was first isolated from the Chinese drug "Ching-feng-teng," *Sinomenium acutum* Redh *et* Wils. (Menispermaceae) (2), and later from *Cassytha pubescens* R. Br. (Lauraceae) (3), *Croton flavens* L. (Euphorbiaceae) (4), *Corydalis pallida* var. *tenuis* (Fumariaceae) (5), and *Cocculus carolinus* D.C. (Menispermaceae) (6).

This is the first reported occurrence of sinoacutine in *Glaucium contortuplicatum*. Recently, it was reported to be present in *G. flavum* (7). Sinoacutine had been synthesized (8) and found to elicit mild antitussive properties (9).

EXPERIMENTAL[1]

A quantity (20 kg) of the air-dried and powdered whole plant was extracted with hot petroleum ether (bp 30–60°) for 36 hr and then with hot methanol for 72 hr. The alcoholic extract, after charcoal treatment and concentration, gave a large amount of inorganic nitrates and an alkaloid, dicentrine nitrate (1), which were removed by filtration. After trituration of the filtrate with acetone to remove sugars, the acetone-soluble portion was evaporated to dryness *in vacuo*, dissolved in water, made basic with ammonium hydroxide to pH 9, and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate, and chromatographed over a column containing neutral aluminum oxide S.

Elution with benzene and treatment of the dried residue with acetone yielded colorless prisms of sinoacutine (150 mg), mp 194–197° dec. [lit. (4) mp 197–199°]; $[\alpha]_D^{26.5}$ −117.64° (c 0.81, ethanol) [lit. (4, 6) $[\alpha]_D^{23}$ −115.0° (c 1.03, ethanol), $[\alpha]_D^{28}$ −115.8° (c 1.0, ethanol)]; UV: λ_{max} (methanol) 214 (log ϵ 4.22), 240 (4.21), and 280 (3.72) nm [lit. (6) UV: λ_{max} (ethanol) 214 (log ϵ 4.40), 245 (4.30), and 280 (3.80) nm]. Major absorptions in the IR spectrum were at ν_{max} (KBr) 3450 (broad OH stretch), 1670, 1640, and 1610 cm^{-1} (cyclohexadienone system).

The NMR spectrum in deuterochloroform showed a three-proton singlet at δ 2.45 (NCH_3), two three-proton singlets at δ 3.73 and 3.84 (2-OCH_3), two one-proton singlets at δ 6.36 and 7.56 (two olefinic protons), and two barely resolved proton peaks at δ 6.70 and 6.75 (two aromatic protons), which were in close agreement with the literature values (6, 10). In addition, the four methylene bridge protons were located as two triplets centered at δ 1.16 and 3.66 as determined by irradiation.

Deuterium exchange experiments permitted the assignment of a broad peak centered at δ 6.2 to the phenolic proton. The remaining one methine and one methylene protons appeared between δ 2.0 and 3.4 but were complex and somewhat obscured by the NCH_3 signal; no further attempt was made to assign these protons. The mass spectrum gave a molecular ion at *m/e* 327, which was also the base peak, followed by other significant peaks at *m/e* 312 ($M^+ - CH_3$, 40%), 299 (M^+ − CO, 22%), and 284 (M^+ − CO—CH_3, 55%). The fragmentation pattern is similar to that of salutaridine (11), the enantiomer of sinoacutine.

The isolate was compared with a reference sample of sinoacutine[2] and was found identical in all respects (IR, UV, mass spectrometric, TLC, melting point, and R_f data), and a mixed melting point was not depressed.

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[1] The plant material was collected near Shiraz, Iran, in April 1971 and was identified as *Glaucium contortuplicatum* Boiss. (Papaveraceae) by M. H. Bokhari, Department of Biology, Pahlavi University. A voucher specimen (DPH-G2740) representing the collection was deposited in the Department of Pharmacology Herbarium, Pahlavi University, Shiraz, Iran.
UV spectra were taken using a Beckman model D-G grating spectrophotometer. IR spectra were taken using a Beckman model IR 18 A spectrophotometer. Mass spectra were determined using a single-focusing Hitachi Perkin-Elmer model RMU-6D mass spectrometer, operating at 70 ev. NMR spectra were taken using a Varian T-60 instrument operating at 60 MHz. Optical rotations were recorded using a Carl Zeiss optical polarimeter. The melting-point and mixed melting-point determinations were made using a Kofler hot-stage melting-point apparatus.

[2] A reference sample of sinoacutine was provided by D. J. Slatkin, Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh.

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Effect of Sodium Salicylate on Hamster Cells *In Vitro*

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Abstract □ Doses of sodium salicylate greater than 100 μg/ml increased the generation time of baby hamster kidney (BHK 21) cells in culture from 16 to 35 hr. Exposure to similar doses of salicylate for 18–44 hr resulted in a marked reduction of RNA synthesis. The species of RNA synthesized in the presence of sodium salicylate appeared to be similar to those synthesized by normal cells in the absence of sodium salicylate. Sodium salicylate did not alter the oxidative phosphorylation of BHK cells.

Keyphrases □ Sodium salicylate—effect on hamster kidney cells in culture, generation time, RNA synthesis □ Cell culture—hamster kidney cells, effect of sodium salicylate on generation and RNA synthesis □ RNA synthesis—hamster kidney cells in culture, effect of sodium salicylate □ Analgesics—sodium salicylate, effect on hamster kidney cells in culture, generation time, RNA synthesis

Aspirin is used to treat various rheumatic diseases. Sodium salicylate has been used as a probe for *in vitro* studies because its effect was similar to that of aspirin on DNA synthesis by stimulated lymphocytes *in vitro* (1). Sodium salicylate inhibited the *in vitro* protein, RNA, and DNA syntheses of human lymphocytes responding to phytohemagglutinin or antigen (1–3). Since great variability has been observed in the suppressive effect of sodium salicylate on human lymphocytes, a genetically defined cell line of baby hamster kidney (BHK 21) cells was selected as a model system to study the effect of salicylate on mammalian cells *in vitro*.

EXPERIMENTAL

Baby hamster kidney cells[1] (BHK 21) were grown as monolayers in a carbon dioxide incubator at 37° using Dulbecco modified Eagle's medium[2] supplemented with 10% fetal calf serum[3] and 10% tryptose phosphate broth[2]. The growth of cells was monitored by harvesting the monolayer after trypsinization and counting the cells using a hemocytometer[4]. Viability was determined by exclusion of 0.1% trypan blue dye.

Total "early labeled" RNA synthesis was determined by exposing the cells to ^{3}H-uridine[5] (2 μCi/ml) for 30 min. At the end of the exposure, sodium azide (0.2%) in saline was added and the cells were harvested and divided into two aliquots. One aliquot was used to measure the radioactivity incorporated in the 10% trichloroacetic acid-insoluble fraction; the other aliquot was used to determine the total DNA by the method of Seibert (4). RNA synthesis was measured as radioactivity incorporated per milligram of DNA.

To determine the effect of sodium salicylate[6] on the type of RNA molecules synthesized, exponentially growing cells were exposed to sodium salicylate (200 μg/ml) for 18 hr. After this incubation time, ^{3}H-uridine (2 μCi/ml) was added for 30 min. Then cells were harvested and mixed with cells that had been grown in the absence of sodium salicylate and similarly exposed to 0.5 μCi/ml of ^{14}C-uridine[5].

RNA was extracted from the cells as previously described (5). The sedimentation profile of the radioactive RNA was determined by layering an aliquot on top of a sucrose linear gradient [5–40% sucrose in 0.01 *M* sodium acetate (pH 5.1), 0.1 *M* NaCl, and 0.001 *M* edetate disodium] in a tube fitting the SW41 rotor of an ultracentrifuge[7].

After 17 hr of centrifugation at 4°, 0.5-ml fractions were collected from the top of the tube using a density gradient fractionator[8]. The fractionator recorded the optical density at 260 nm continuously. To each fraction, 1 ml of cold 20% trichloroacetic acid was added, followed by 5 ml of cold 10% trichloroacetic acid to precipitate RNA. The resulting sediment was collected over a 0.45-μm filter[9], and the acid-precipitable radioactivity was determined.

Determination of the oxidative phosphorylation and the hexose monophosphate shunt was adapted from the method previously described (6). In brief, 3 ml of medium containing 750,000 BHK cells was dispensed into 100-ml serum bottles[10], and sodium salicylate was added to triplicate flasks in a final concentration of 200 μg/ml. Control bottles were prepared containing fewer cells (500,000/3 ml) so that the numbers of cells in each category (sodium salicylate and control) would be equivalent at the end of the 24-hr incubation period.

After 24 hr of incubation, the medium was decanted from all bottles. The cell monolayer was washed with 0.85 *M* phosphate-buffered saline containing 1000 μg/ml of dextrose. To each culture, 3.0 ml of saline–dextrose containing 200 μg/ml of sodium salicylate or saline was added. ^{14}C-Glucose, labeled in the C-1 or C-6 position, was then added (0.5 μCi/ml). The bottles were immediately capped with rubber stoppers, from which were suspended ampuls containing 1.0 ml of a quaternary ammonium hydroxide[11].

[1] Obtained from Dr. C. Basilico.
[2] Grand Island Biological, Grand Island, N.Y.
[3] Flow Laboratories, Rockville, Md.
[4] Newbauer.
[5] New England Nuclear, Boston, Mass.
[6] Merck Chemical Division, Merck & Co., Rahway, N.J.
[7] Spinco L265, Beckman Instrument Co., Fullerton, Calif.
[8] Instrumentation Specialties Co., Lincoln, Neb.
[9] Millipore, Bedford, Mass.
[10] Bellco Glass, Inc., Vineland, N.J.
[11] Hyamine hydroxide, Packard, Downers Grove, Ill.