Alkaloids of *Glaucium flavum* Grantz, Populations Isfahan and Kazerun

A. SHAFIEE [x], I. LALEZARI, S. LAJEVARDI, and F. KHALAFI

Abstract □ *Glaucium flavum* Grantz, population Isfahan, contained four major alkaloids: dicentrine (0.8%), bulbocapnine (0.42%), protopine (0.35%), and salutaridine (0.2%). *G. flavum* Grantz, population Kazerun, contained four major alkaloids: dicentrine (1.4%), bulbocapnine (0.5%), *O*-methylflavinantine (0.5%), and salutaridine (0.3%); it also contained two minor alkaloids, protopine and α-allocryptonine. *O*-Methylflavinantine was found for the first time in the Papaveraceae.

Keyphrases □ *Glaucium flavum* Grantz—populations Isfahan and Kazerun, major alkaloids identified □ Alkaloids—identified in *Glaucium flavum* Grantz, populations Isfahan and Kazerun

Recently, in a continuation of a research program dealing with the chemotaxonomic studies of Iranian wild Papaveraceae (1–4), the major alkaloids of *Glaucium flavum* Grantz, population Ghom, were reported (5). In this work, the alkaloids of *G. flavum* Grantz, populations Isfahan (south) and Kazerun[1] (southwest of Iran), are reported. Although *Glaucium* species growing in Ghom, Isfahan, and Kazerun are morphologically similar and were identified as *G. flavum* Grantz, careful chemical studies revealed that they are independent chemotypes.

EXPERIMENTAL[2]

Plant Material—The whole aerial parts were collected during May, air dried in the shade, further dried at 60° to a constant weight, and powdered so that all material passed a mesh not greater than 0.5 mm.

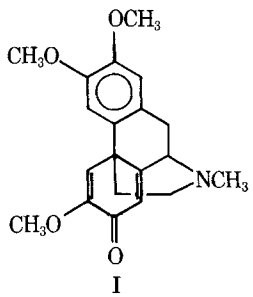
Extraction—Powdered plant material, 200 g, was moistened with 200 ml of 15% ammonium hydroxide solution, stirred with 600 ml of chloroform at room temperature for 1 hr, and filtered. The extraction was repeated four times. After the evaporation of the solvent, the residue was extracted with 100 ml of 5% sulfuric acid. The solution was filtered and extracted with petroleum ether (3 × 20 ml) to remove the colored material. The aqueous layer was made alkaline with 15% ammonium hydroxide solution and extracted with chloroform (4 × 50 ml).

Evaporation of the solvent gave a residue of crude alkaloids. The crude extract was subjected to preparative TLC (silica gel $HF_{254+366}$, thickness 1 mm, plate size 20 × 20 cm). Two solvent systems were used for the separation of alkaloids: A, ethyl acetate–methanol–ammonia (85:10:5); and B, petroleum ether–chloroform–diethylamine (70:20:10). Resolved components were detected under 254-nm UV light. Appropriate zones were removed from the plates, and the components were extracted with chloroform–methanol (80:20). The solvent was evaporated under reduced pressure, and the alkaloids were crystallized from an appropriate solvent and characterized.

RESULTS AND DISCUSSION

The following alkaloids were isolated from *G. flavum* population Isfahan:

1. Dicentrine, R_f 0.6 (Solvent System A) and 0.44 (Solvent System B); yield 1.6 g (0.8%); mp 166–169° [lit. (5) mp 166–169°].



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2. Bulbocapnine, R_f 0.65 (Solvent System A) and 0.32 (Solvent System B); yield 0.8 g (0.4%); mp 199–200° [lit. (5) mp 199–200°].

3. Salutaridine, R_f 0.42 (Solvent System A) and 0.15 (Solvent System B); yield 0.4 g (0.2%); mp 197–199° [lit. (5) mp 197–199°].

[The melting points, mixed melting points, IR, UV, NMR, and mass spectral data of these alkaloids were identical with those reported (5).]

4. Protopine, R_f 0.71 (Solvent System A) and 0.5 (Solvent System B); yield 0.7 g (0.35%), mp 205–207° [lit. (6) mp 207°]; mixed melting point with an authentic sample 205–207°. Its NMR spectrum was identical with that reported previously (7).

From *G. flavum* population Kazerun, in addition to dicentrine (1.5%), bulbocapnine (0.5%), salutaridine (0.3%), and protopine (minor alkaloid), the following alkaloid was also isolated:

α-Allocryptonine, R_f 0.59 (Solvent System A) and 0.47 (Solvent System B); mp 160–161° [lit. (8) mp 160–161°]; mixed melting point with an authentic sample 160–161°. Its spectral data were similar to those reported previously (9, 10).

In addition, another alkaloid was also isolated [R_f 0.35 (Solvent System A) and 0.15 (Solvent System B)]. This alkaloid was an oil; UV: λ_{max} (ethanol) 282 and 238 nm; IR ($CHCl_3$): 1666 (C=O), 1642, and 1621 cm^{-1}; NMR ($CDCl_3$): δ 1.93 (m, 2H), 2.44 (s, 3H, NCH_3), 2.49–3.66 (m, 5H), 3.82 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.30 (s, 1H), 6.53 (s, 1H), 6.70 (s, 1H), and 6.95 (s, 1H); mass spectrum: *m/e* 341 (M^+), 326 ($M^+ - 15$), 313 ($M^+ - 28$), 298 ($M^+ - 43$), 282, and 149.

Anal.—Calc. for $C_{20}H_{23}NO_4$: C, 70.38; H, 6.74; N, 4.11. Found: C, 70.45; H, 6.83; N, 4.26.

The hydrochloride had a melting point of 195–196° (ethanol). The methiodide had a melting point of 223–225° (methanol) [lit. (11) mp 223–225°].

The spectral data (UV, IR, NMR, and mass) as well as the TLC data of this alkaloid (I) were identical with an authentic sample of *O*-methylflavinantine.

The alkaloid profiles of *G. flavum* Grantz, populations Ghom, Isfahan, and Kazerun, indicate that they are independent chemotypes. The major alkaloids of Ghom are dicentrine, bulbocapnine, and salutaridine. *G. flavum* population Isfahan also has protopine as a major alkaloid. Protopine could not be detected in *G. flavum* population Ghom. Finally, *G. flavum* population Kazerun also has *O*-methylflavinantine, which was not present in the other populations. A literature survey revealed that *O*-methylflavinantine was found in *Nemuaron vieillardii* (Monimiaceae) (12) and *Rhigiocarja racemifera* Miers (Menispermaceae) (13).

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[1] The plants were identified by Professor K. Hummel, Tubingen University. Herbarium samples were deposited in the Herbarium of the College of Pharmacy, Tehran University.

[2] Melting points were taken with a Koffler hot-stage microscope and are uncorrected. UV spectra were recorded on a Varian Techtron 635 instrument. NMR spectra were taken with a Varian T 60A instrument, using tetramethylsilane as the internal standard. Mass spectra were recorded on CH5 spectrometer at Arya-Mehr University. IR spectra were obtained with a Leitz model III spectrograph. Protopine and α-allocryptonine were obtained from Koch-Light Laboratories, Colnbrook SL3 OBZ, Buckinghamshire, England.

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[x] To whom inquiries should be directed.

Rapid Determination of Procainamide and Its *N*-Acetyl Derivative in Human Plasma by High-Pressure Liquid Chromatography

ORVILLE H. WEDDLE and WILLIAM D. MASON [x]

Abstract □ A rapid, specific, high-pressure liquid chromatographic method for the determination of procainamide and its *N*-acetyl derivative in plasma was developed. The procedure is fast enough (15 min from receipt of blood to reporting value) to be used for emergency determinations. The sensitivity, precision, and accuracy are sufficient for routine monitoring of therapeutic levels in patients. The assay is specific enough to be valid in the presence of a number of drugs and dietary substances present in clinical samples.

Keyphrases □ Procainamide and *N*-acetyl derivative—high-pressure liquid chromatographic analyses, human plasma □ High-pressure liquid chromatography—analyses, procainamide and *N*-acetyl derivative, human plasma □ Cardiac depressants—procainamide and *N*-acetyl derivative, high-pressure liquid chromatographic analyses, human plasma

Procainamide (I) is an antiarrhythmic compound which demonstrates marked intersubject variability in absorption and elimination (1). Its *N*-acetyl metabolite (II) in humans (2) has antiarrhythmic activity comparable to that of I (3) but demonstrates an even greater variability in plasma concentration with a given I dosage schedule. Methods of monitoring plasma I concentrations that do not also determine II are not adequate.

Recently, several methods for the simultaneous determination of I and II were reported. A fluorometric method required more than 4 ml of blood for a duplicate determination and involved reextraction into acid, addition of alkali, and two fluorescence measurements (4). No mention was made of the possible interference of the fluorescent base quinidine, which would be extracted under these conditions and might be present in patients receiving procainamide.

A GLC method was reported for I and II determinations in urine samples (5), but no mention was made of its application to blood samples. Another GLC method for the determination of both I and II in blood, urine, and saliva samples was reported (6). However, each duplicate analysis probably required 1 hr or more because of a three-stage acid–base extraction, evaporation, and the 23.5-min retention time for II.

A quantitative TLC approach to the problem was developed (2). The reported standard deviations of 9% for I and 11% for II reflect a well-known difficulty of quantitative TLC.

None of the reported methods claimed the combination of small sample size, rapidity, specificity, and precision desirable for routine patient monitoring. This paper describes a method for performing a duplicate analysis in 16 min on as little as 300 μl of whole blood with sufficient accuracy, precision, and specificity for clinical samples.

EXPERIMENTAL

Materials—UV grade hexane, 2-propanol, methanol, 1-butanol, and chloroform[1] and reagent grade concentrated ammonium hydroxide[2] were used as obtained. High purity samples of I and II[3] were used in the preparation of standards.

Apparatus—A high-pressure liquid chromatograph[4] was equipped with a septumless injection port[5] and a fixed wavelength (280 nm) UV absorption detector[6]. The detector was operated at 0.005 and 0.01 absorbance unit full scale.

Chromatographic Parameters—The mobile phase was prepared by diluting concentrated ammonium hydroxide 10:1 with distilled water. One milliliter of this solution was added to 1 liter of 2-propanol and 1 liter of hexane, and the solution was diluted to 5 liters with methanol. The mobile phase was pumped at 5.0 ml/min (3500 psi) through a stainless steel column (4 mm i.d. × 30 cm) packed with a high efficiency bonded-phase absorption packing[7].

Analytical Procedure—Two 50-μl aliquots of each plasma sample were placed in 1.5-ml disposable centrifuge tubes[8]. To each tube was added 10 μl of 5 *N* NaOH solution and 600 μl of an organic extraction solution containing 20% 1-butanol, 20% chloroform, and 60% hexane by volume. The two tubes, together with any other samples being run at the same time, were shaken for 30 sec at 1350 oscillations/sec[9] and then centrifuged for 30 sec at 15,000 rpm[10].

[1] Burdick and Jackson Laboratories, Muskegon, Mich.
[2] Mallinckrodt Chemical Works, St. Louis, Mo.
[3] E. R. Squibb & Sons.
[4] Model 6000A, Waters Associates, Milford, Mass.
[5] Model U6K, Waters Associates, Milford, Mass.
[6] Model 440, Waters Associates, Milford, Mass.
[7] μBondapak CN, Waters Associates, Milford, Mass.
[8] Brinkmann Instruments, Westbury, N.Y.
[9] Eppendorf Micro Shaker, Brinkmann Instruments, Westbury, N.Y.
[10] Eppendorf Micro Centrifuge, Model 3200, Brinkmann Instruments, Westbury, N.Y.