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Note

Isolation of jamaicin and lisetin and their determination in *Piscidia erythrina* L. by high-performance liquid chromatography

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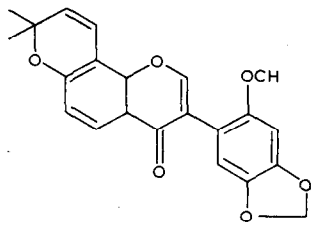
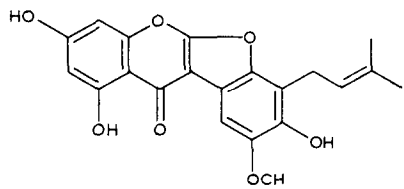
The Jamaican Dogwood, *Piscidia erythrina* L., has been the subject of numerous investigations concerned with the analgesic and insecticidal properties of the root-bark as well as its pharmacodynamic properties[1,2]. Considerable variation in the products isolated from this plant source has been noted[3]. However, the isoflavones jamaicin and lisetin were confirmed as characteristic constituents[4–6].



Jamaicin



Lisetin

A number of high-performance liquid chromatographic (HPLC) methods have been reported for the analysis of flavonoids[7,8]. However, owing to the particular difficulty in obtaining jamaicin and lisetin, so far there is no analytical procedure for the evaluation of these isoflavones in *Piscidia erythrina* L.

Therefore, we report in the present paper a simple procedure for the isolation of jamaicin and lisetin and their determination in *Piscidia erythrina* L. by reversed-phase HPLC.

EXPERIMENTAL

Equipment

Ultraviolet (UV) spectra were recorded on Pye Unicam Model SP 1700, infrared (IR) spectra on Beckman Acculab 3 Model and nuclear magnetic resonance (NMR) spectra on Hitachi Perkin-Elmer Model R248 spectrophotometers. A Water Assoc. analytical liquid chromatograph equipped with a Waters Lambda Max Model UV detector was used throughout this investigation.

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Reagents

Methanol was of HPLC grade (Chromasolv; Riedel-De Haën); water was distilled in glass and then passed through a 0.45-μm membrane filter (Type HA, Millipore). All other reagents and solvents were analytical grade.

Thin-layer chromatography (TLC)

Merck silica gel 60 F_{254} (0.25 mm) plates were used. The fractions containing jamaicin and lisetin were developed with chloroform–ethyl acetate (95:5). Jamaicin and lisetin were detected by irradiation at 254 nm or by spraying with 10% methanolic $AlCl_3$ [R_F = 0.47 (jamaicin), 0.23 (lisetin)].

HPLC

The analytical column was a Waters μBondapak C_{18} (30 cm × 3.9 mm I.D.); a pre-column (Waters Assoc., Part. No. 84550), 2 cm × 4 mm I.D., packed with Bondapak/Corasil (37–50 μm) was used to extend the life of the analytical column. A mobile phase of water–methanol (25:75) adjusted to pH 2.8 with 10% phosphoric acid was used at a flow-rate of 2.0 ml/min (170 atm at room temperature). The effluent was monitored at 254 nm and the signal recorded with a Model 730 Data Module (Waters Assoc.).

Calibration curves

Stock solutions of jamaicin and lisetin were prepared by dissolving each compound in methanol (200 μg/ml). Standard solutions (2.5–60 ng/μl) were prepared diluting the stock solutions in the mobile phase. Replicate injections of 10 μl were made for each sample.

Sample preparation

The extracts of *Piscidia erythrina* L. (1:2, *i.e.*, 1 part of extract corresponds to 2 parts of plant tissue) were obtained from commercial sources (Sochibo, Boulogne, France; Milanfarma, Milan, Italy and Sir, Mede, Italy). A 5-ml volume of each sample was added to 5 ml of distilled water and extracted with ethyl acetate (3 × 10 ml). The organic extracts were dried over sodium sulphate and evaporated to dryness under vacuum. The residue was dissolved in 10 ml methanol. A 5-μl aliquot of each solution was injected into the liquid chromatograph.

Isolation of jamaicin and lisetin

Piscidia erythrina L. extracts (1:2), 1500 g, were extracted three times with 500 ml of chloroform. After evaporation of the solvent to dryness, the residue was extracted with hot benzene (3 × 50 ml), and the combined extracts were reduced to low volume.

This benzene solution was chromatograph on a silica gel column (40 cm × 50 mm I.D.). 100-ml Fractions were collected by elution with chloroform following TLC on silica gel F_{254} with chloroform–ethyl acetate (95:5).

Only fractions 5 and 6 (main spot at R_F = 0.47) and 14–17 (main spot at R_F = 0.23) were collected and they yielded crude solids after evaporation of the eluent.

Crystallization from ethanol of the residue from fractions 5 and 6 gave jamaicin (5) (55 mg), m.p. 193°C, λ_{max} (ethanol) 231, 263 and 306 nm. IR (KBr, cm^{-1}):

1647, 1634, 1597, 1575, 1398, 1266, 1117, 1038, 933. NMR (deuterochloroform): δ 8.1 (d, J = 9.5 Hz), 7.97 (s), 6.98–6.68 (m), 6.0 (s), 5.73 (d, J = 9.5 Hz), 3.74 (s) and 1.48 (s).

Crystallization from methanol of the residue from fractions 14–17 yielded lisetin (6) (40 mg), m.p. 285°C, λ_{max} (ethanol) 216, 258, 284 and 338 nm, IR(KBr, cm^{-1}): 3560, 3240, 1653, 1620, 1518, 1398, 1076, 1018, 780. NMR ([$^2H_6$]dimethyl sulphoxide): δ 12.9 (s), 7.2 (s), 6.54 (d, J = 2 Hz), 6.30 (d, J = 2 Hz), 5.32 (m), 3.90 (s), 3.5 (m), 1.81 (s) and 1.69 (s).

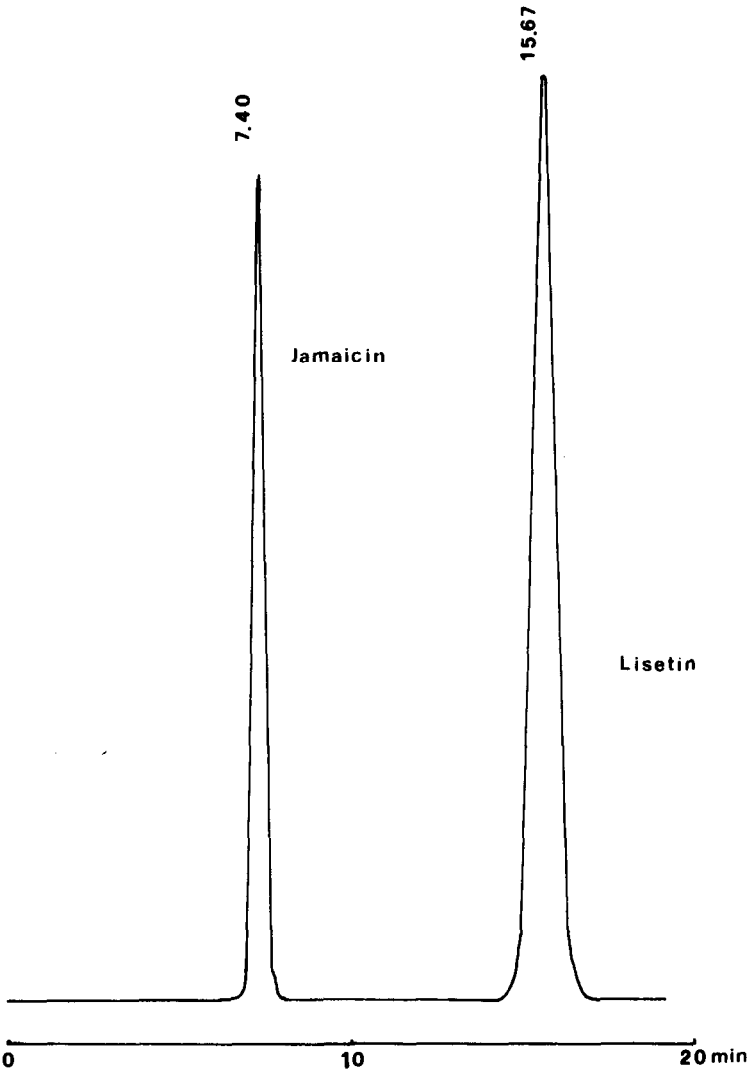

Fig. 1. High-performance liquid chromatogram of jamaicin and lisetin. Eluent: methanol–water (75:25) (pH 2.8). Column: μBondapak C_{18}. Flow-rate: 2.0 ml/min. Detection: UV 254 nm.

RESULTS AND DISCUSSION

Jamaicin and lisetin were isolated from *Piscidia erythrina* L. extracts (1:2) by means of column chromatography followed by crystallization. The structures of both compounds were confirmed by UV, IR and NMR spectroscopy.

We required to find a set of HPLC operating parameters for the separation of

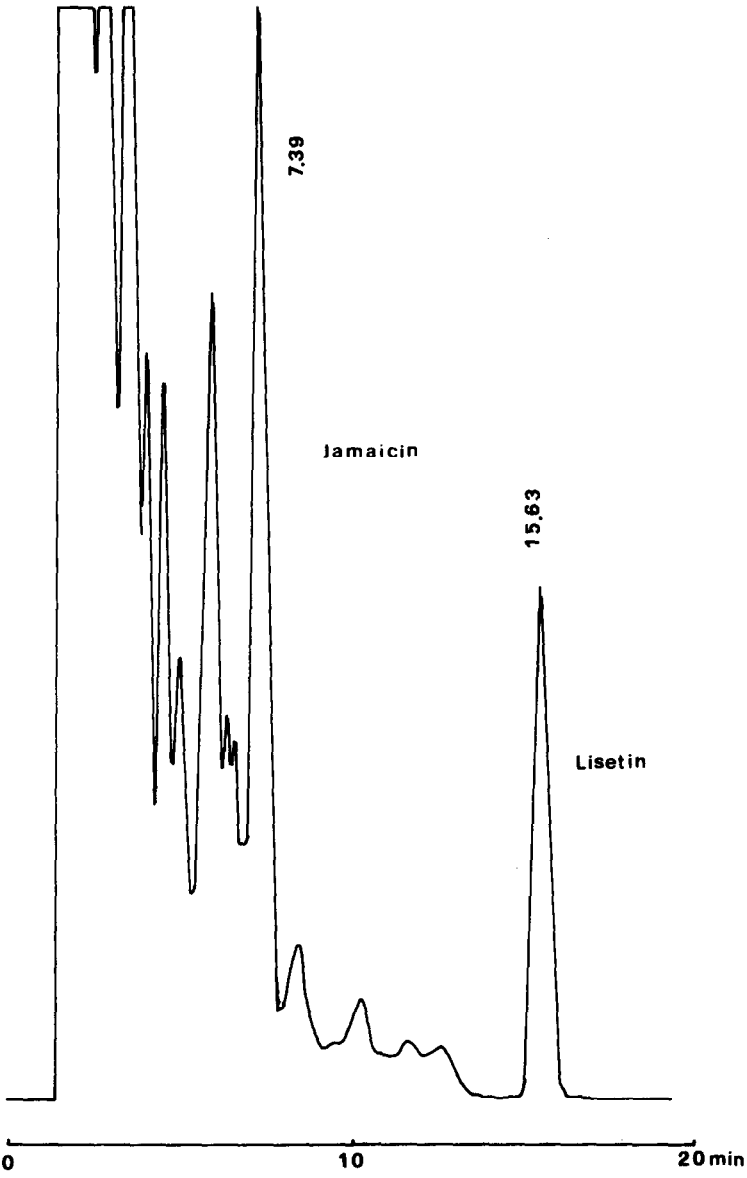

Fig. 2. High-performance liquid chromatogram of *Piscidia erythrina* L. extracts. Chromatographic conditions as in Fig. 1.

the multiple components and for the detection of jamaicin and lisetin even at very low levels. Reversed-phase HPLC on an octadecyl column was suitable for these purposes. Jamaicin and lisetin were best separated using an isocratic elution mode with methanol–water (75:25) (pH 2.8) as eluent and gave retention times of 7.40 and 15.67 min, respectively. (Fig. 1). A flow-rate of 2.0 ml/min (170 atm at room temperature) was the most satisfactory, since it allowed separations in less than 17 min.

As shown in Fig. 2, jamaicin and lisetin were well separated from the other components of *Piscidia erythrina* L. extracts. The identification of the resolved peaks was carried out using the co-injection technique with authentic samples. The standard deviations of the retention times for jamaicin and lisetin were 0.36% and 0.48%, respectively, indicating that the separation was highly reproducible. The determination of jamaicin and lisetin was performed by external standardization. The linearity of the detector response was checked for both compounds by injecting known amounts of pure samples, integral measurements of the peaks areas and plotting these areas against amount injected. Linear relationships between peak areas and the amounts of jamaicin and lisetin were found over the range investigated (up to 500 ng) and can be expressed by the equations $y = 7.610x + 10.867$; $r = 0.994$ (for jamaicin), and $y = 9.674x + 0.800$; $r = 1.000$ (for lisetin).

The contents of both isoflavones in the examined extracts were considerably different (from 0.003 to 0.012%). Under the experimental conditions used, the limit of detection for jamaicin and lisetin was 5 ng.

In conclusion, the proposed method gives a rapid, sensitive and reproducible quantification of jamaicin and lisetin in *Piscidia erythrina* L. extracts.

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