PHYTOCHEMICAL INVESTIGATION OF *TEPHROSIA CANDIDA*: HPLC SEPARATION OF TEPHROSIN AND 12a-HYDROXYROTENONE

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Tephrosia candida (Roxb.) DC. (Papilionaceae) is a shrub; an extract of its seeds has been tested for insecticidal properties and found to be quite effective (1). Previous phytochemical studies have yielded a glycoside with anticancer activity (2), candidin (3), 6a,12a-dehydrodeguelin (4), pongachin (4), and a corresponding chalcone (4). In the present paper, we report the isolation from the seeds and the separation of tephrosin and 12a-hydroxyrotenone by hplc; caffeic acid, β-sitosterol, and its glucoside were also isolated. These compounds have been isolated for the first time from this genus, and the mixture of tephrosin and 12a-hydroxyrotenone has been resolved for the first time using hplc.

EXPERIMENTAL

Dried, coarsely ground seeds (2 kg) of *T. candida* (identified by Dr. C.R. Babu, Associate Professor, Department of Botany, University of Delhi, no voucher specimen available) were defatted with hot hexane and then thoroughly extracted with C_6H_6 and EtOH successively. The combined hexane and C_6H_6 extracts (15 g) were chromatographed over Si gel (500 g). Elution with C_6H_6/EtOAc in order of increasing polarity yielded pongachin as yellow needles [20 mg, mp 148–150°, lit. (4) 145–148°] and candidin as yellow crystals [2 mg, mp 198–200°, lit. (3) 193–196°]. The concentrate of the EtOH extract was macerated with Et_2O and the Et_2O solubles (30 g); cc over Si gel (1 kg) using hexane/EtOAc with increasing polarity as eluents yielded in order β-sitosterol (20 mg), 6a,12a-dehydrodeguelin [30 mg of pale yellow crystals, mp 229°, lit. (6) 224.5–230.5°], a further crop of pongachin (25 mg), the mixture M (100 mg), a further crop of candidin (20 mg), caffeic acid [10 mg, mp 197°, lit. (5) 200°; the diacetyl derivative, mp 195°, lit. (5) 198°] and β-sitosterol glucoside (50 mg).

The mixture M crystallized from EtOAc/hexane as colorless crystals, mp 146°. Upon development in various solvent systems, on tlc it showed only one spot. Its color reactions and λ max (MeOH) 235, 271, and 293 nm indicated the presence of a rotenoid skeleton. Its ^{1}H-nmr data [two singlets for four methoxyls at δ 3.68 (6H) and 3.86 (6H), two multiplets at δ 4.54 (8H) and 6.49 (7H), two doublets at δ 7.66 (1H, J=8 Hz) and 7.76 (1H, J=8 Hz), a doublet at δ 5.57 (1H), a singlet at δ 5.02 (2H), three singlets, each for 3H at δ 1.36, 1.42, and 1.72, a multiplet at δ 3.12 (2H) and a triplet at δ 5.20 (1H)] suggested M to be a mixture of two compounds having the same basic skeleton but with one possessing additionally a 2,2-dimethylchromene moiety and the other a furan moiety. The eims showed peaks at *m/z* 410 $[M]^+$, 208, 207, 203, 193, and 181. From these observations, mixture M was inferred to consist of tephrosin and 12a-hydroxyrotenone; this was supported by the ^{1}H-nmr and ms data of its acetyl derivative. The same mixture has earlier been isolated from *Derris urucu* (7) and *Tephrosia praecans* (8) and has not been resolved. We, for the first time, have separated the mixture by hplc on a Vydac RP-18 10-μm (10 nm pore size) column (Hesperia, CA), the solvents being 25% iPrOH and 75% H_2O at a flow rate of 1.0 ml/min. Retention time on the 4×250 nm column was 10.5 min for 12a-hydroxyrotenone and 12.5 min for tephrosin.

Full details of hplc separation and identification of compounds are available on request from the senior author.

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[1]Since deceased.