Chemical Constituents of Gentianaceae IX: Natural Occurrence of Erythrocentaurin in *Enicostemma hyssopifolium* and *Swertia lawii*

S. GHOSAL[x], A. K. SINGH, P. V. SHARMA, and R. K. CHAUDHURI

Abstract □ Erythrocentaurin was isolated from two gentianaceous plants—*viz.*, *Enicostemma hyssopifolium* (Willd.) Verd. (synonymous with *E. littorale*) and *Swertia lawii* Burkill., as a native compound. Its natural occurrence was demonstrated for the first time by using a modified isolation procedure. The identity of the compound was established by chemical transformations and spectral (UV, IR, NMR, and mass spectra) evidence. Phylogenetic significance of the cooccurrence of erythrocentaurin and a number of structurally related monoterpene alkaloids in *E. hyssopifolium* is discussed.

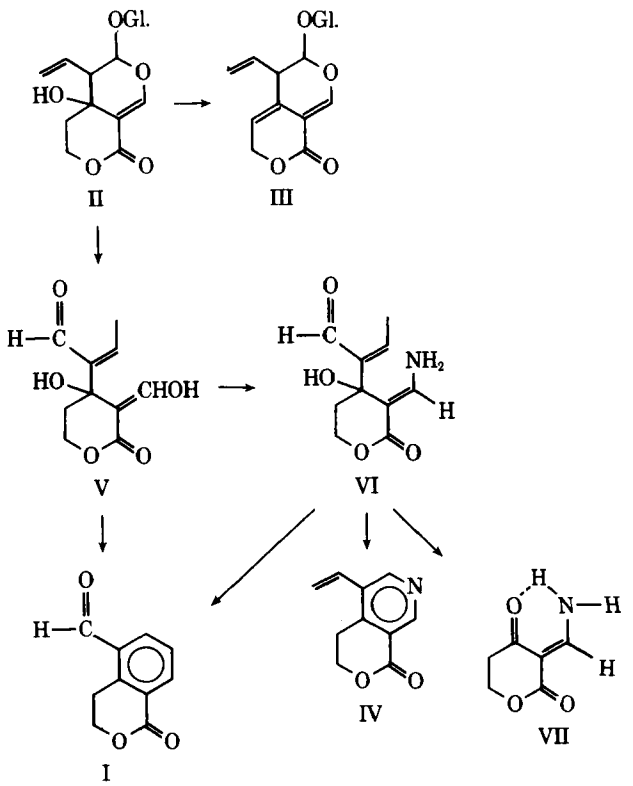
Keyphrases □ Erythrocentaurin—isolation and identification from *Enicostemma hyssopifolium* and *Swertia lawii* □ *Enicostemma hyssopifolium*—natural occurrence of erythrocentaurin and structurally related monoterpene alkaloids □ *Swertia lawii*—natural occurrence of erythrocentaurin and gentianine □ Enicoflavine—spontaneous transformation into erythrocentaurin, gentiocrucine, and gentianine

Erythrocentaurin (I) was first isolated by Méhu (1) from commercial samples of *Erythrea centaurium*. Subsequently, it was obtained (2) by the enzymatic hydrolysis of swertiamarin (a native glucoside of *Swertia japonica*) and of erytaurin (a native glycoside of *Er. centaurium*) (3). The structure of I was established later by Kubota and Tomita (4), who suggested it to be an artifact formed by the hydrolysis of swertiamarin (II). Compound I was also isolated (5) from *Gentiana lutea*. But this time, the process of isolation, involving alkalization of the root extracts of *G. lutea*, did not eliminate the possibility of its formation from swertiamarin present in the plant in appreciable quantities. Thus, the question of whether erythrocentaurin is an artifact or a native compound in these plants remained unsettled. Evidence is presented in this report establishing that erythrocentaurin is a native compound in two gentianaceous plants—*viz.*, *Enicostemma hyssopifolium* (Willd.) Verd. (synonymous with *E. littorale* Blume) and *Swertia lawii* Burkill. (synonymous with *S. corymbosa* Wight). In addition, the cooccurrence of erythrocentaurin with a number of structurally related monoterpene alkaloids in *E. hyssopifolium* (6) is appraised in the light of a common biogenetic sequence.

RESULTS AND DISCUSSION

E. hyssopifolium and *S. lawii* whole plants were separately extracted with petroleum ether. The petroleum ether extracts were processed for neutral, basic, and glycosidic constituents. Although it seemed unlikely that a weakly polar extract would contain appreciable quantity of any strongly polar constituent, *e.g.*, swertiamarin (II) and gentiopicroside (III), tests for these compounds were carried out to dispel any gap in the finding. The absence of swertiamarin and gentiopicroside in the petroleum ether extracts and isolation of erythrocentaurin from the neutral fraction established that erythrocentaurin occurs as such in these two species. The identity of erythrocentaurin was established by chemical transformations and spectral (UV, IR, NMR, and mass spectra) evidence. The NMR and mass spectral data of this compound were not reported previously. The basic fraction of the two plant extracts afforded gentianine (IV). Previous studies showed (7) that gentianine can exist as a native alkaloid in small quantities, but the relatively high yield of gentianine, reported in many plants, results from the action of ammonia on nonnitrogenous precursors, *e.g.*, swertiamarin (II) and gentiopicroside (III). The present report of isolation of gentianine from *E. hyssopifolium* and *S. lawii*, without involving treatment with ammonia, should be regarded as additional evidence in favor of labeling it as a natural alkaloid. The aglucone (V) of swertiamarin, which remains to be isolated, is an accepted intermediate to gentianine (5, 7, 8). Compound V could also act as an intermediate to erythrocentaurin in plants. The transformation of V to I would involve only minor molecular rearrangement followed by the elimination of water.

Recently, isolation of two new monoterpene alkaloids, enicoflavine (VI) and gentiocrucine (VII), from *E. hyssopifolium* was reported (9). Enicoflavine was shown to transform spontaneously into gentianine, gentiocrucine, and erythrocentaurin. Upon electron impact (in the mass spectrometer), it was converted into gentianine and gentiocrucine (6). If the premise is granted that



Scheme I—Hypothetical route to genesis of monoterpene alkaloids and erythrocentaurin in E. hyssopifolium

enicoflavine is formed from the same aglucone (V) in *E. hyssopifolium*, then a plausible common sequence of genesis of the congener compounds would be as shown in Scheme I.

The conversion of V into VI would involve simple amination (the source of nitrogen *in vivo* is, however, not known) at the hydroxymethylene group. The subsequent transformation of VI into VII would proceed *via* retroaldolization; the accompanying crotonaldehyde was also detected (6). Further details of these findings will be reported.

EXPERIMENTAL[1]

Extraction of *E. hyssopifolium*[2]—Air-dried and milled whole plants (1.6 kg), collected from the local area and properly identified[3], were extracted (16 hr) under reflux in a soxhlet apparatus with petroleum ether (bp 60–80°). The petroleum ether extract was concentrated to about 400 ml, and a portion of the concentrate (300 ml) was extracted with aqueous citric acid (12.5%, 200 ml) using a mechanical stirrer (8 hr). The acidic aqueous layer was kept for further processing for erythrocentaurin. The other portion (100 ml) of the petroleum ether extract was extracted with ethyl acetate (3 × 100 ml). The combined ethyl acetate extracts were washed with water, dried (anhydrous sodium sulfate), and concentrated (about 25 ml). The concentrate was passed through a column of alumina[4] (24 × 1.8 cm), and the column was eluted with methanol (500 ml). The methanol eluate was concentrated (20 ml) and examined for the monoterpene glucosides, swertiamarin (11) and gentiopicroside (11), by analytical TLC in the presence of authentic markers. The two glucosides were absent in this fraction. Only a weakly polar constituent, R_f 0.8 [*n*-butanol–acetic acid–water (4:1:2)], was detected in the methanol eluate.

Isolation of Erythrocentaurin (I)—The aqueous citric acid layer was extracted with ether (3 × 100 ml). The combined ether extracts were washed, dried, and concentrated (about 20 ml). The ether concentrate, after sitting, afforded brown crystals of erythrocentaurin (27 mg). It crystallized from acetone–petroleum ether (1:1) as colorless needles, mp 132–133° [lit. (11) mp 140–141°]; R_f 0.40 [benzene–chloroform (100:20)], 0.79 [*n*-butanol–acetic acid–water (4:1:2)]; UV: λ_{max} (ethanol) 224 (log ϵ 4.28) and 290 nm (3.09); IR: ν_{max} (mineral oil) 1718 (conjugated lactone C=O) and 1695 cm^{-1} (conjugated —CHO); mass spectroscopy *m/e* 176 (M^+, 100%) and significant fragment ion peaks at *m/e* 148 (14), 147 (17), 131 (8), 120 (40), 119 (43), 118 (46), 117 (11), and 105 (8); NMR (100 MHz): δ ($CDCl_3$) 3.6 and 4.6 [two 2H triplets, the large downfield shift due to $-CH_2-CH_2-$ moiety attached to a hetero atom, associated with the functionality —O—C(=O)—], 7.65 (1H, t, J = 8 Hz), 8.15 (1H, dd, J = 8 Hz, J' = 1.6 Hz), 8.42 (1H, dd, J = 8 Hz, J' = 1.6 Hz) (three aromatic protons), and 10.3 (1H, s, —CHO).

Anal.—Calc. for $C_{10}H_8O_3$: C, 68.18; H, 4.54. Found: C, 67.74; H, 4.73.

Direct comparison with the reported (11) erythrocentaurin was not possible since that material was not available. After sitting and being exposed to light, the compound gradually turned reddish brown and showed several spots with analytical TLC.

The semicarbazone, prepared in the usual way, crystallized from ethanol as colorless needles, mp 217–218° [lit. (11) mp 219–220°].

Anal.—Calc. for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.72; N, 18.02. Found: C, 56.41; H, 4.98; N, 17.83.

Isolation of Gentianine (IV)—The clarified acidic aqueous layer, after separation of erythrocentaurin, was made basic with sodium bicarbonate. The liberated bases were extracted with ether (3 × 100 ml). The combined ether extracts were washed, dried, and evaporated. The residue crystallized from petroleum ether–benzene as colorless needles (12 mg), melting point and mixed melting point with authentic gentianine 80°; co-TLC with authentic gentianine showed a single spot, R_f 0.68 [*n*-butanol–acetic acid–water (4:1:2)]; λ_{max} (ethanol): 220 (log ϵ 4.39), 245 inflection (3.88), and 275–278 nm (3.20).

Anal.—Calc. for $C_{10}H_9NO_2$: C, 68.57; H, 5.14; N, 8.00. Found: C, 68.83; H, 4.90; N, 7.57.

Extraction of *S. lawii*[5]—Air-dried and milled whole plants (1.2 kg) were extracted with petroleum ether, and the extracts were processed in the same way as described for *E. hyssopifolium* when erythrocentaurin (24 mg) and gentianine (17 mg) were obtained from the neutral and basic fractions, respectively.

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[x] To whom inquiries should be directed.

[1] The general directions are same as reported in a recent paper (10).

[2] The plant material was supplied by Mr. B. Singh, Varanasi, India. A herbarium specimen has been preserved at the Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

[3] Through the courtesy of Dr. C. S. P. Rao, Department of Botany, Faculty of Sciences, Banaras Hindu University.

[4] Brockmann activity grade about IV.

[5] The plant material was supplied by Soil Conservation, Research Training and Demonstration Centre, Ootacumund, the Nilgiris, India. A voucher specimen has been deposited at the Department of Pharmaceutics, Banaras Hindu University.