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DHURRIN, (−)-CATECHIN, FLAVONOL GLYCOSIDES AND FLAVONES FROM *CHAMAEBATIA FOLIOLOSA*

ADOLF NAHRSTEDT, PETER PROKSCH* and ERIC E. CONN†

Institut für Pharmazeutische Biologie und Phytochemie der Universität, D-4400 Münster, F.R.G.; *Institut für Pharmazeutische Biologie der Technischen Universität, D-3300 Braunschweig, F.R.G.; †Department of Biochemistry and Biophysics, University of California, Davis, CA 95616, U.S.A.

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Abstract—The cyanogenic glucoside dhurrin has been obtained in low amounts from the aerial parts of *Chamaebatia foliolosa*. Main flavonoids of the tissue were quercetin 3-*O*-glucoside, 3-*O*-galactoside, 3-*O*-rhamnoside and 3-*O*-arabinoside, whereas the flavones apigenin and hispidulin were detected as resin flavonoids deposited on the epidermis. The rare (−)-catechin was a minor constituent of the tissue extract.

INTRODUCTION

Chamaebatia foliolosa Benth. (mountain misery) is an evergreen shrub of 20–60 cm with pinnate glandular-pubescent sticky leaves and an aromatic odour. It occurs in open pine forests in the foothills of middle and northern California [1]. Despite its wide distribution in this region there are no phytochemical reports on this plant. Tests with Feigl–Anger paper [2] showed weak cyanogenesis for the tips; a quantitative determination of HCN liberated with β-glucosidase from almonds showed 0.32 μmol/g fr. wt for the shoots and 0.28 μmol/g fr. wt for the blossoms. The seeds were acyanogenic (less than 0.01 μmol/g). The following describes the cyanogenic compound and some flavonoids which were obtained during purification.

RESULTS AND DISCUSSION

Lyophilized aerial plant material was extracted with benzene and subsequently with methanol–ethyl acetate. The latter extract was filtered over polyamide and the cyanogenic eluent was chromatographed using droplet counter-current chromatography (DCCC). Flavonol 3-*O*-glycosides were obtained during concentration of the cyanogenic DCCC fraction as a precipitate, which was further purified on Sephadex LH-20 and by TLC to yield quercetin 3-*O*-glucoside as well as its 3-*O*-galactoside, its 3-*O*-rhamnoside and its 3-*O*-arabinoside. They were identified by comparative TLC with reference compounds [3] on silica gel and after hydrolysis by cochromatography of the aglycones and the sugars with reference compounds. Direct hydrolysis of the above mentioned precipitate additionally revealed kaempferol in small amounts suggesting low concentrations of some kaempferol glycosides.

The residual DCCC fraction was further purified on silica gel and by using HPLC on RP-18 to give *ca* 5 mg of a pure compound which was identical with dhurrin (2-β-D-glucopyranosyloxy-2-(4-hydroxy)phenyl-2*S*-acetonitrile) in its behaviour on TLC, GLC, and its ^{1}H NMR spectrum [4, 5].

The first DCCC fractions yielded a relatively apolar and, upon detection on TLC plates, phenolic substance which was isolated by further column chromatography on silica gel and Sephadex LH-20. The ^{1}H NMR and ^{13}C NMR of the unknown established the presence of catechin with the 2,3-*trans* configuration as indicated by the *trans* coupling constant of 7.8 Hz of H_2 with H_3, and the resonance position of C_2 of 82.8 ppm [6, 7]. $[\alpha]_D^{20°}$ was −14.3° in methanol ($c = 0.42$), thus indicating (−)-catechin [8] with the 2*S*,3*R* configuration.

The flavones apigenin and hispidulin were identified in the benzene extract of the lyophilized plant material by comparative TLC with authentic compounds. Several years ago both flavones were detected in the external resin of *Ch. foliolosa* obtained by rinsing the leaves; both compounds were identified by their UV and MS spectra [P. Proksch and P. A. Rundel, unpublished].

Ch. foliolosa is the second member of the Rosaceae which contains dhurrin as the cyanogenic glucoside; recently dhurrin was identified from *Cercocarpus ledifolius* and other *Cercocarpus* sp. [5, 9]. The occurrence of dhurrin is exceptional since the Rosaceae are mainly characterized by phenylalanine derived prunasin and amygdalin with the *R*-configuration at C-2 [10]. However, the amount in *Ch. foliolosa* is low and, assuming a defence role for the cyanogenic glycosides [11], it seems that this plant in its dry habitat has evolved other constituents for defence. One group of these may be the mixture of quercetin 3-*O*-glycosides which occur in high yield in the leaf tissue; mixtures of these compounds are reported to exhibit deterrent activities against herbivores [12]. The occurrence of external leaf flavonoids is typical for plants living in semiarid habitats

[13]. Among these the flavones represent a large group with fungicidal, bactericidal and virustatic activities [14]. External apigenin and hispidulin have not previously been identified in a member of the Rosaceae [15]. It is noteworthy that the flavonol 3-*O*-glycosides had been found exclusively in tissue extracts, but the free flavones seem to be surface constituents only. Whereas (+)-catechin occurs frequently in nature, (−)-catechin is a rare compound [16]. It was isolated from catechu (obtained from *Acacia catechu*) together with (+)-catechin probably as a product of isomerization [17]. The optical rotation of $[\alpha]_D^{20°} - 14.3°$ when compared to reported data of $-16.8°$ [8] reveals that the compound was almost pure. Recently (−)-catechin 7-*O*-glucoside and its (+)-isomer were isolated from the bark of *Rhaphiolepis umbellata* (Rosaceae) and commercial rhubarb (Polygonaceae) [16].

EXPERIMENTAL

Plant material and clean up. The plant material was collected east of Placerville, California in September 1985, immediately frozen and lyophilized. 160 g dry material was first refluxed with benzene, then extracted with 1:1 mixture of MeOH–EtOAc. The latter was taken to dryness, the residue suspended in H_2O, centrifuged and the concd supernatant filtered with H_2O over polyamide (2 × 25 cm). The first 250 ml contained the cyanogenic fraction. This was concd and used for DCCC [18] with $CHCl_3$–MeOH–H_2O 35:65:40 in the descending mode using 300 glass tubes (0.2 × 400 mm). Fractions were monitored by TLC (silica gel; EtOAc–MeCOEt–H_2O–FormOH 7:5:1.5:5:0.2) and cyanide test paper [2]. The (−)-catechin fraction (I) eluted within 90–120 ml, the cyanogenic fraction (II) eluted within 270–370 ml.

Concentration of II yielded a high amount of precipitate (III). CC of the supernatant of II on silica gel (2 × 50 cm) with EtOAc–MeEtCO–H_2O–AcOH (7:3:5:1) yielded a cyanogenic fraction within 70–170 ml which, after concn, was further purified by HPLC on RP-18 (0.8 × 25 cm) with H_2O–MeOH–MeCN–AcOH 85:5:9.5:0.5 (4.5 ml/min det. at 260 nm). The cyanogenic substance was eluted after 5.5 min.

CC of fraction I on silica gel (1.5 × 20 cm) with EtOAc–MeOH–AcOH 7:3:0.05 yielded (−)-catechin within 85–115 ml.

Identification of dhurrin. The cyanogenic sample obtained by HPLC was lyophilized and the residue analysed by TLC and GC of the TMSi-derivative as described in [5]. The ^{1}H NMR spectrum of the residue in acetone-d_6 was identical to that presented in [5].

The flavonol glycosides (III) were obtained by concn of II and further purified on Sephadex LH-20 (1 × 80 cm) with MeOH as eluent and by following semipreparative TLC with reference compounds on silica gel with the above mentioned system. The zones were scraped off, hydrolysed with 1 N HCl and the aglycones extracted with EtOAc. The aglycones were chromatographed on TLC plates (cellulose; HOAc–H_2O 2:3; det. Naturstoffreagens) in comparison to reference compounds. Comparative TLC of the sugars was made on silica gel; MeCN–CS_2–H_2O 85:5:1 (det. with anisaldehyde–H_2SO_4).

Identification of the flavones. The benzene extract was comparatively chromatographed on TLC on polyamide using benzene–MeEtCO–MeOH–H_2O (55:23:20:2) (det. Naturstoffreagens); R_f values: apigenin 0.35, hispidulin 0.68.

Identification of (−)-catechin. ^{1}H NMR (acetone-d_6, δ-scale in ppm, Bruker WM 400): 6.7–6.9 (3H; 2′,5′,6′); 6.02 (H_6; *d*, 2.1); 5.87 (H_8; *d*, 2.2); 4.54 (H_2; *d*, 7.8); 3.98 (H_3; *m*); 2.86 (H_4; *dd*, 16.0, 5.4); 2.52 (H_4; *dd*, 16.0, 8.5). This spectrum is almost identical with that of (+)-catechin in [6] but the reversed assignment of H_6 and H_8 in this paper was unambigiously revealed by CH-correlation and CH-long-range correlation spectra [7].

^{13}C NMR spectrum (acetone-d_6). 157.85, 157.57, 156.94 (C 5,7,8a), 146.23 (3′,4′), 132.32 (1′), 120.06 (6′), 116.15 (5′), 115.33, (2′), 100.92 (4a), 96.42 (6), 95.59 (8), 82.87 (2), 68.48 (3), 28.48 (4). The spectrum is almost identical to that of (+)-catechin in [6] but the assignments of C_6 and C_8 were reversed (see ^{1}H NMR).

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REFERENCES

1. Munz, P. A. and Keck, D. D. (1970) *A California Flora*, p. 780. University of California Press, Berkeley.
2. Tantisewie, B., Ruijgrok, H. W. L. and Hegnauer, R. (1969) *Pharm. Weekblad* **104**, 1341.
3. Marby, T. J., Markham, K. R. and Thomas, M. R. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
4. Nahrstedt, A. (1981) in *Cyanide in Biology* (Vennesland *et al.*, eds) p. 146. Academic Press, London.
5. Nahrstedt, A. and Limmer, S. (1982) *Phytochemistry* **21**, 2738.
6. Nonake, G.-I., Ezaki, E., Hayashi, K. and Nishioka, I. (1983) *Phytochemistry* **22**, 1659.
7. Wray, V. unpublished.
8. *The Merck Index*, 10th edn., no. 1883. Rahway, 1983.
9. Nahrstedt, A. unpublished.
10. Fikenscher, L. H., Hegnauer, R. and Ruijgrok, H. W. L. (1981) *Planta Med.* **41**, 313.
11. Nahrstedt, A. (1985) *Pl. Syst. Evol.* **150**, 35.
12. Harborne, J. B. (1979) in *Herbivores, Their Interaction with Secondary Plant Metabolites* (Rosenthal, G. A. and Janzen, D. H., eds) p. 645. Academic Press, New York.
13. Wollenweber, E. (1984) in *Biology and Chemistry of Plant Trichomes* (Rodriquez, E., Healey, P. L. and Mehta, I., eds) p. 53. Plenum Press, New York.
14. Wollenweber, E. (1985) *Pl. Syst. Evol.* **150**, 83.
15. Wollenweber, E. and Dietz, V. H. (1981) *Phytochemistry* **20**, 869.
16. Karrer, W., Cherbuliez, E. and Eugster, C. H. (1977) *Konstitution und Vorkommen der organischen Pflanzenstoffe*. Erg. Bd. 1, No. 1760. Birkhäuser, Basel.
17. Freudenberg, K., Karimullah and Steinbrunn, G. (1935) *Liebigs Ann. Chem.* **518**, 37.
18. Hostettmann, K. (1980) *Planta Med.* **39**, 1.