Phytochemistry, Vol. 24, No. 4, pp. 831–833, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00
© 1985 Pergamon Press Ltd.

DICHOTOSIN AND DICHOTOSININ, TWO ADAPTOGENIC GLUCOSYLOXY FLAVANS FROM *HOPPEA DICHOTOMA**

SHIBNATH GHOSAL, DINESH K. JAISWAL, SUSHIL K. SINGH and RADHEY S. SRIVASTAVA

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-221005, India

(*Revised received* 20 *September* 1984)

Key Word Index—*Hoppea dichotoma*; Gentianaceae; roots; glucosyloxy flavans; dichotosin, (2*S*)-7,4′-dimethoxy-5-*O*-β-D-glucupyranosylflavan; dichotosinin; 7,3′,4′-trimethoxy-5-*O*-β-D-glucopyranosylflavan; diffutin; 7-hydroxy-3′,4′-dimethoxy-5-*O*-β-D-glucopyranosylflavan; central nervous sytem (CNS) active agents; adaptogens.

Abstract—From the roots of *Hoppea dichotoma*, collected before flowering, two new naturally occurring glucosyloxyflavans, dichotosin and dichotosinin, have been isolated and characterized by means of comprehensive spectral analyses, chemical transformation and synthesis of the aglucone of dichotosin. This is the first report of dichotosinin from a natural source. Additionally, one known glucosyloxyflavan, diffutin, earlier reported in another Gentianaceae species (*Canscora diffusa*) also has now been isolated from this species. The glucosyloxyflavans, individually and in combination, produced varying degrees of adaptogenic (anti-stress–anti-anxiety) activity in animal models. This observation is consistent with the use of the plant extract as a nerve tonic in Ayurvedic medicine.

INTRODUCTION

Hoppea dichotoma (Gentianaceae) is used in Ayurvedic medicine in the treatment of haemorrhoids, in cardiac dropsy and as a nerve tonic. Some years ago, we reported [1] the isolation and characterization of 11 polyoxygenated xanthones, two *C*-glucosylflavones, two glucosyloxyflavanones and four triterpenes from the whole plant of *H. dichotoma* collected at flowering. A reinvestigation of this species was considered necessary since an ethyl acetate extract of the roots, collected before flowering, exhibited pronounced adaptogenic (anti-stress–anti-anxiety) activity in laboratory animals. Consequently, chemical investigation of the ethyl acetate extract, based on its biological activity, was undertaken to isolate and identify the CNS active component(s). The findings constitute the subject of this paper.

RESULTS AND DISCUSSION

A combination of column and low pressure chromatography of the ethyl acetate extract of defatted roots of *H. dichotoma* afforded three glucosyloxyflavans, two of which were obtained for the first time from a natural source and have been named dichotosin and dichotosinin, and the third, a previously reported one, diffutin, from *Canscora diffusa* [2]. The quantities were sufficient for their complete chemical characterization and psychopharmacological screening. Details of the characterization of the two new compounds only is described here.

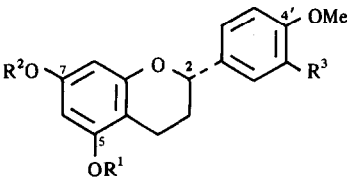
Dichotosin

The compound, $C_{23}H_{28}O_9 \cdot H_2O$ (by elemental analyses of the compound and $[M]^+$ of the tetraacetate), exhibited UV and IR spectra characteristic of glucosyloxyflavans (devoid of any oxygen substituent in the hetero ring) [3]. The 90 MHz ^{1}H NMR spectrum of the compound, in deuteriodimethylsulphoxide, exhibited signals for six aromatic protons, the coupling patterns and constants of which suggested the substitution as in **1**. Additionally, there were signals for four methylene protons ($-CH_2 \cdot CH_2-$), one methine proton associated with a benzyl ether system (H-2 of flavans) [3], two aromatic methoxyl groups, a glucosyl anomeric proton and several hydroxyl groups. As expected for a glucosyloxyflavan, dichotosin fragmented in the electron impact mass spectrometer before giving a $[M]^+$ peak. However, the significant fragment ion peaks corresponded with the aglucone (**4**) and glucose moieties. The additional fragment ion peaks, of appreciable abundance, at *m/z* 153 and 165 were ascribed to the ion fragments **6** and **7**, respectively (by accurate mass measurement) [3]. Dichotosin formed a tetra-acetate (**5**) with acetic anhydride and triethyl amine. The ^{1}H NMR spectrum of the tetra-acetate suggested that all its acetyl groups were associated with the sugar moiety. Hydrolysis of dichotosin with emulsin afforded the aglucone (**4**), mp 167–169°; $[\alpha]_D$ −11.3° (MeOH) and D-glucose. The aglucone was unstable in methanol and underwent autooxidation to give a red polymeric compound in addition to other products. Finally, reduction of a reference sample of (2*S*)-5-hydroxy-7,4′-dimethoxyflavanone (**8**) with sodium borohydride in acetic acid afforded **4**, thereby establishing the stereochemistry at C-2 of dichotosin. Thus, the (2*S*)-7,4′-dimethoxy-5-*O*-β-D-glucopyranosylflavan structure (**1**) was assigned to dichotosin. Dichotosin has not been encountered naturally before nor has it been prepared before synthetically.

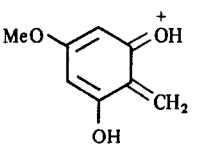
Dichotosinin

The compound, $C_{24}H_{30}O_{10} \cdot H_2O$ (by elemental analyses of the compound and $[M]^+$ of the tetra-

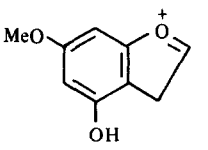
*Part 32 in the series: "Chemical Constituents of Gentianaceae". For Part 31 see ref. [2].



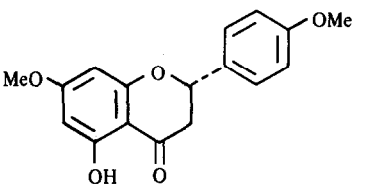
1 $R^1=\beta$-D-Glc, R^2 = Me, R^3 = H
2 $R^1=\beta$-D-Glc, R^2 = Me, R^3 = OMe
3 $R^1=\beta$-D-Glc, R^2 = H, R^3 = OMe
4 $R^1=R^3$ = H, R^2 = Me
5 $R^1=\beta$-D-Glc $(OAc)_4$, R^2 = Me, R^3 = H



6 *m/z* 153



7 *m/z* 165



8

acetate) showed physical and spectral properties indistinguishable from those of 7-*O*-methyldiffutin, prepared from diffutin by methylation with diethyl ether–methanol–diazomethane [2]. Dichotosinin was, therefore, assigned the 7,3′,4′-trimethoxy-5-*O*-β-D-glucopyranosylflavan structure (**2**). This is the first report of its natural occurrence.

Dichotosin (**1**) and dichotosinin (**2**) are the fifth and sixth examples of naturally occurring glucosyloxyflavans. The other four glycosyloxy- (glucosyloxy/xylosyloxy-) flavans have been found to be distributed in six plant families [3], viz. Ericaceae, Gentianaceae, Leguminosae, Liliaceae, Myristicaceae and Santalaceae.

The glucosyloxyflavans (**1–3**) exhibited significant adaptogenic activity, in laboratory animals using a variety of tests [(a) gross behaviour, (b) spontaneous motor activity (SMA), (c) rotarod performance, (d) restraint ulcers, (e) swimming stress endurance, and (f) the ability to sensitize the pituitary–adrenal axis which is regarded as a contributory factor in preparing animals against stress] [4, 5] accepted for arriving at such a conclusion. Thus, intraperitoneal (i.p.) administration of the total extract (20 mg/kg·day for 3 days) produced significant adaptogenic activity in albino mice and rats. Diffutin (**3**) (10–20 mg/kg·day) was shown [2] to produce a moderate adaptogenic effect in these animals. A 1:1 combination of **1**, **2** or **3**, with their aglucones (in i.p. doses of 10 mg/kg·day for 3 days) produced pronounced adaptogenic activity in albino mice and rats. The synergism exhibited by the glucosyloxyflavans and the corresponding aglucones has precedence in other series of bioactive phenolic glycosides and aglycones [6]. The details of the biological evaluation of dichotosin and dichotosinin will be reported elsewhere.

*The plant material was collected in August 1981 and September 1982 from the Varanasi District of Uttar Pradesh, India, and properly identified [1]. A voucher specimen of each has been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University.

EXPERIMENTAL

The general procedures were those reported recently [7].

Extraction procedure. Dried and powdered roots of *H. dichotoma** Willd. (0.8 kg) were continuously extracted (Soxhlet) with petrol (bp 60–80°) for 30 hr. The defatted material was then extracted (30 hr), in succession, with $CHCl_3$ and EtOAc. The EtOAc extract was concd and chromatographed over silica gel (30 × 2 cm) with $CHCl_3$ and $CHCl_3$–MeOH (99:1) as eluants. The appropriate $CHCl_3$–MeOH eluates (monitored by analytical HPLC and by TLC using the benzidine–metaperiodate test for glycosides) were combined, concd and kept at room temp. for several days when a brown solid ppted.

Diffutin (**3**). The brown solid was repeatedly crystallized from MeOH to give diffutin as cream coloured needles (1.2 g), mp 144–145°; R_f 0.35 (silica gel G/UV_{254}, $CHCl_3$–MeOH, 9:1); UV λ_{max}^{MeOH} nm: 222 sh, 270; $[\alpha]_D^{22}$ −44.8° (MeOH; *c* 0.7); ^{1}H NMR [$(CD_3)_2SO$]: δ9.2 (1H, exchangeable with D_2O, OH-7), 7.1 (3H, *m*, H-2′, H-5′, H-6′), 6.18 (1H, *d*, *J* = 2.5 Hz, H-8), 5.98 (1H, *d*, *J* = 2.5 Hz, H-6), 5.2–4.6 (5H), 3.8 (6H, OMe). The physical and spectral properties of the compound were indistinguishable from those of diffutin [2].

The MeOH mother liquors were combined after separation of diffutin and evaporated to give a brown gummy material (0.48 g), consisting of a mixture of glucosyloxyflavans in which **1** and **2** were the major constituents. A portion (*ca* 0.1 g) of the gummy material was dissolved in MeOH–H_2O (1:1) and applied to a Lobar RP8 column. Elution was carried out with the same solvent (1.4 l.). Fractions (20 ml) were collected and monitored by analytical HPLC (μ-Bondapak C_{18} column 440/250 nm detector MeOH–H_2O, 4:1, as eluant).

Dichotosin (**1**). The first peak of appreciable intensity on HPLC appeared in fraction 12. The next eight fractions were combined and evaporated *in vacuo* to give dichotosin as a cream coloured powder (42 mg) with no definite mp; R_f 0.45; $[\alpha]_D^{22}$ −34.5° (MeOH; *c* 0.5), UV λ_{max}^{MeOH} nm (logε): 222 (4.26), 272 (3.20), 278 sh (3.08); IR ν_{max}^{Nujol} cm^{-1}: 3450 (*br*, OH), 1618, 1600 (*br*, sugar moiety), 1500, 1178, 1032, 1005, 890; ^{1}H NMR [$(CD_3)_2SO$]: δ7.21 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 6.88 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.12 (1H, *d*, *J* = 2.5 Hz, H-8), 5.98 (1H, *d*, *J* = 2.5 Hz, H-6), 4.6–5.22 [*br m*, on exchange with D_2O the multiplicity collapsed to δ4.9 (1H, *d*, *J* = 9 Hz, glucosyl anomeric H) and δ4.7 (1H, *q*, H-2)] [3], 3.8 (3H, *s*, OMe-7), 3.78 (3H, *s*, OMe-4′), 3.5 (*br*, sugar protons plus OH), 2.0–2.7 (4H, *m*, methylene protons); MS

m/z (rel. int., %): 286 (75), 271 (7), 177 (7), 165 (11), 163 (7), 162 (5), 153 (18), 152 (11), 150 (7), 135 (10), 134 (100), 121 (7), 109 (12), 91 (8); *m/z* of **6** by accurate mass measurement: 153.053, $C_8H_9O_3$ requires *m/z* 153.054; *m/z* of **7** by accurate mass measurement: 165.054; $C_9H_9O_3$ requires *m/z* 165.054.

Acetylation of dichotosin with Ac_2O–Et_3N, at room temp. under dry conditions, afforded the tetra-acetate derivative which crystallized from MeOH as colourless needles, mp 147–149°; ^{1}H NMR [$(CD_3)_2SO$]: δ7.24 (2H, *d*, *J* = 8.5 Hz, H-2', H-6'), 6.86 (2H, *d*, *J* = 8.5 Hz, H-3', H-5'), 6.44 (1H, *d*, *J* = 2.5 Hz, H-8), 6.42 (1H, *d*, *J* = 2.5 Hz, H-6), 4.8–5.3 (5H, *m*), 3.8 (6H, OMe), 1.95–2.0 (12H, OAc); MS *m/z* (rel. int., %): 616 $[M]^+$ (2.5), 331 (18), 286 (85), 271 (9), 169 (42), 134 (100).

Hydrolysis of dichotosin. Dichotosin (12 mg) was emulsified with emulsin (10 mg), in aq. NaOAc–HOAc buffer (pH *ca* 5; 10 ml). Work-up in the usual way afforded the aglucone, dichotosidin (**4**) (5 mg), mp 167–169°; $[\alpha]_D^{22}$ −11.3° (MeOH; *c* 0.34); UV λ_{max}^{MeOH} nm: 222, 278, 282; the UV spectrum of a MeOH soln of **4**, on keeping at room temp. for 2–3 days, exhibited increasing absorption with λ_{max} *ca* 400 nm; evaporation of the MeOH soln yielded a red polymeric compound along with other products of varying polarities (TLC, analytical HPLC); (**4**) MS *m/z* (rel. int. %): 286 $[M]^+$ (100), 165 (11), 153 (14), 150 (7), 134 (88), 119 (5), 91 (7). The sugar component present in the aq. hydrolysate was identified as D-glucose by GC of the alditol acetate according to ref. [8].

Synthesis of dichotosidin (**4**). (2*S*)-5-Hydroxy-7,4'-dimethoxyflavanone (**8**) (7 mg) (kindly provided by Professor N. Adityachaudhury, B.C.K. University, West Bengal), in HOAc (5 ml), was reduced with $NaBH_4$ (20 mg) added in portions over 4 hr; the reaction was monitored by TLC. Dilution with H_2O and extraction of the slightly acidified soln with Et_2O gave dichotosidin (**4**) (3 mg). The identity of the synthetic and natural flavans was established by direct comparison (co-TLC, HPLC, MS).

Dichotosinin (**2**). Fractions 37–50 from the Lobar chromatography were combined and evaporated *in vacuo* to give dichotosinin as a glassy solid (28 mg); R_f 0.38; ^{1}H NMR [$(CD_3)_2SO$]: δ7.08 (3H, *m*, H-2', H-5', H-6'), 6.05 (1H, *d*, *J* = 2.5 Hz, H-8), 5.95 (1H, *d*, *J* = 2.5 Hz, H-6), 4.6–5.2 (5H, *m*), 3.82 (6H, OMe), 3.80 (3H, OMe); MS *m/z* (rel. int., %): 316 (78), 301 (10), 286 (8), 177 (9), 165 (14), 164 (100), 153 (18), 150 (9), 121 (7), 91 (5). (Found: C, 58.3; H, 6.8. $C_{24}H_{30}O_{10}\cdot H_2O$ requires C, 58.6; H, 6.4%.) The tetra-acetate derivative, prepared with Ac_2O–Et_3N at room temp. under dry conditions, crystallized from MeOH as colourless needles, mp 158–160°; MS *m/z* (rel. int., %): 646 $[M]^+$ (3.5), 331 (18), 316 (72), 271 (12), 169 (24), 43 (100). Direct comparison of dichotosinin with 7-*O*-methyldiffutin [2] established that the two compounds were identical.

Adaptogenic activity testing. Pharmacological studies were conducted on albino mice (18–25 g) and albino rats (80–120 g). The animals were fed on a standard pellet diet. All expts were carried out at 28 ± 2°. The test compounds were dissolved in H_2O. The control group of animals received only the vehicle (H_2O). In each set of expts, 10 animals were used. Statistical analyses were done by the Student *t*-test and the χ^2-test at appropriate places. In the primary observational study, the test compounds were administered intraperitoneally (i.p.) and the changes in the gross behaviour and motor activity (SMA) were recorded at 15, 30, 60 and 120 min. Definite signs of CNS stimulation and increased motor activity were observed for the test compound treated groups. The changes reached a peak by 30 min which were sustained up to 60 min and then gradually declined by 120 min. The swimming stress endurance and the stress induced ulceration of the stomach expts were carried out according to refs [4, 5]. The test compounds potentiated the swimming endurance of albino rats by 60–130% over the control groups. Likewise, treatment with **1** and **2**, in combination with their aglucones, provided 40–100% protection against stress stomach ulceration. Stress is also known to lower the serum corticosterone level. Administration of the test compounds, in multiple doses (for 3 days), increased the serum corticosterone level by 1.5–1.8-fold. In another set of expts, the distribution of corticosterone in the brain was estimated. There was a decrease in the pituitary corticosterone level by *ca* 1.6-fold and an increase in the cerebrum corticosterone level by *ca* 1.7-fold. The results were statistically significant.

Acknowledgements—We are indebted to Dr. Nitya Nand, Director, CDRI, Lucknow, and Dr. S. K. Bhattacharya, Department of Pharmacology, Banaras Hindu University, Varanasi, for some of the analytical and pharmacological data. S.K.S. is grateful to the University Grants Commission, New Delhi, for a research fellowship.

REFERENCES

1. Ghosal, S., Jaiswal, D. K. and Biswas, K. (1978) *Phytochemistry* **17**, 2119.
2. Ghosal, S., Saini, K. S. and Sinha, B. N. (1983) *J. Chem. Res.* (*S*) 330; (*M*) 2601.
3. Saini, K. S. and Ghosal, S. (1984) *Phytochemistry* **23**, 2415.
4. Bhattacharya, S. K., Ghosal, S., Chaudhuri, R. K., Singh, A. K. and Sharma, P. V. (1974) *J. Pharm. Sci.* **63**, 1341.
5. Bhattacharya, S. K., Reddy, P. K. S. P., Ghosal, S., Singh, A. K. and Sharma, P. V. (1976) *J. Pharm. Sci.* **65**, 1547.
6. Ghosal, S., Banerjee, S. and Jaiswal, D. K. (1980) *Phytochemistry* **19**, 332.
7. Ghosal, S., Kumar, Y. and Singh, S. P. (1984) *Phytochemistry* **23**, 1167.
8. Ghosal, S. and Saini, K. S. (1984) *J. Chem. Res.* (*S*) 110; (*M*) 965.