Phytochemistry, 1979, Vol. 18, pp. 359–360. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0359 $02.00/0

A NEW FLAVONOL GLYCOSIDE FROM *RUDBECKIA BICOLOR**

P. K. JAUHARI, S. C. SHARMA, J. S. TANDON and M. M. DHAR
Central Drug Research Institute, Lucknow-226001, India

(*Revised received* 1 *August* 1978)

Key Word Index—*Rudbeckia bicolor*; Compositae; 7,8-dimethylherbacetin-3-rhamnoside; 8-hydroxyflavonol.

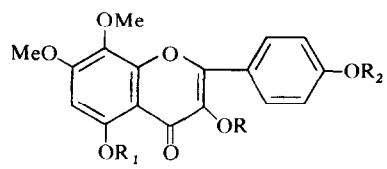
Rudbeckia bicolor Nutt. is a common ornamental, summer-flowering annual. Previous chemical investigations are limited to a report on the presence of gibberellin-like substances [1]. In the present study a new flavonol glycoside 7,8-dimethylherbacetin 3-rhamnoside (**1**) has been characterised from an ethanolic extract of the whole plant. The flavonol glycoside mp 189°, analysed for $C_{23}H_{24}O_{11}$, (M^+ 476). Its IR spectrum displayed a strong absorption band at 1650 cm^{-1} for chelated carbonyl and for C—O stretching between 1090 and 1050 cm^{-1}. The bonded phenol gave rise to absorption at 3380 cm^{-1} and these bands were assignable to its aromatic systems at 1590, 1545, 1480, 800 and 740 cm^{-1}.

The UV spectrum of the glycoside in MeOH showed a much stronger absorption at band II, 273 nm than band I, 333 nm indicating that the sugar residue is at C-3, a conclusion which is further supported by the hypsochromic shift of band I when compared with the aglycone (see below). The bathochromic shift of 53 nm of band I with $AlCl_3$ is a characteristic feature of 5-hydroxy-3-substituted flavonols [2] and the absence of a NaOAc shift indicates that the 7-hydroxyl [3] is substituted. The neutral spectrum of the glycoside showed a pronounced shoulder on the long wave side of band I. This has been correlated with the presence of 4′-hydroxyl in molecules having the 3- or 7-positions substituted [4]. The negative borate reaction indicates the absence of an *o*-dihydroxyl grouping and the bathochromic shift of 69 nm in band I without a decrease in intensity in the presence of NaOMe confirmed the presence of a free 4′-hydroxyl [2].

The glycoside formed a crystalline penta-acetate mp 97° whose NMR spectrum (τ scale) exhibited a sharp doublet at 9.24 (J = 6 Hz), which is a distinguishing feature for rhamnosyl C$\underline{H}_3$. Also the rhamnosyl C-1″ H coupled with the C-2″ $\underline{H}$ (J = 2 Hz) and appeared at 4.53 while the other four sugar protons appeared in the region of 5.32–4.64. The five C$\underline{H}_3$CO resonated as singlets at 8.19, 8.12, 7.92, 7.86 and 7.67 and two sharp C$\underline{H}_3$O singlets appeared at 6.17 and 6.33. The resonance for one aromatic H resonated as a singlet at 3.36 and a pair of *ortho* coupled doublets for four protons of an A_2B_2 system could be seen at 2.88 and 2.27 (J = 9.0 Hz) and indicated the *para* substitution of ring B and only one ring A H. The NMR spectra clearly indicated mono-glycosidation in the molecule.

Acid hydrolysis of the original glycoside yielded an aglycone and rhamnose. The aglycone (**2**) mp 262°, analysed for $C_{17}H_{14}O_7$ (M^+ 330) had ν_{max} 3400 (bonded OH), 1642 (chelated C=O) and 842 cm^{-1} (*p*-substituted phenyl ring). Its UV spectrum had λ_{max} at 272 and 354 nm. A large bathochromic shift of 96 nm with NaOMe degenerated after 10 min indicating that the molecule had free hydroxyls at C-3 and C-4′ [4–6]. The other shifts with diagnostic reagents were similar to those observed for the original glycoside. The aglycone formed a triacetate (**3**) mp 200° whose NMR spectrum showed signals for three C$\underline{H}_3$CO at 7.55, 7.68 and 7.71, for two C$\underline{H}_3$O at 6.03 and 6.16 locating them at C-7 and C-8 positions. The C-6 H now appeared as a singlet at 3.05 and the *para* substituted ring B H resonated as symmetrical doublets centred at 2.2 and 2.8 (J = 8.5 Hz). The aglycone is thus 7,8-dimethoxy-3,5,4′-trihydroxyflavone and the glycoside the corresponding 3-rhamnoside (**1**).

The structure was confirmed by methylation of the glycoside (**1**) with MeI and Ag_2O followed by hydrolysis. The methylated aglycone (**4**), mp 108°, $C_{19}H_{18}O_7$, had λ_{max} 260 and 352 nm. Bathochromic shifts of 62 and 60 nm in presence of $AlCl_3$/HCl and NaOMe, respectively, showed that the C-3 hydroxyl which was glycosylated in (**1**) had become free. Finally this structure was confirmed by methylation of the glycoside with CH_2N_2 followed by hydrolysis which yielded a compound, identical with an authentic sample of tambulin (**5**) (mmp, TLC, IR and UV) [7] kindly supplied by Prof. Asima Chatterji.



1 R_1 = R_2 = H; R = Rha
2 R_1 = R_2 = R = H
3 R_1 = R_2 = R = COMe
4 R_1 = R_2 = Me; R = H
5 R_1 = R = H; R_2 = Me

EXPERIMENTAL

Uncorr. capillary mps are reported. IR, UV and 60 MHz NMR spectra were taken in KBr, MeOH and CCl_4 with TMS as internal standard, respectively. TLC was performed on Si gel G plates and flavones visualized by cerric sulphate spray.

Isolation of glycoside (**1**). Air-dried powdered plant material (2.5 kg) was extracted with 50% EtOH at room temp. and the extract concentrated under red. pres. at a temp. below 50°. The residue obtained was extracted successively with 1 l. each of C_6H_{14}, C_6H_6 and EtOAc. The EtOAc extract (12 g) was concentrated and chromatographed over Si gel (350 g), employing non-polar → polar solvent mixtures. The mixture eluted with EtOAc was fractionated by PLC using EtOH–$CHCl_3$ (1:3) as solvent and a pale yellow glycoside (**1**), mp 189° (MeOH) was obtained. R_f 0.7 (EtOH–$CHCl_3$, 1:3) and 0.11 (C_6H_{14}–$CHCl_3$–MeOH, 14:4:2). (Found: C, 58.20; H, 502. $C_{23}H_{24}O_{11}$ requires: C, 57.98; H, 5.04%). Acetate mp 97°. NMR: 2.27 (*d*, J = 9 Hz, 2H), 2.88 (*d*, J = 9 Hz, 2H), 3.36 (*s*, 1H), 4.53 (*d*, J = 2 Hz, rhamnosyl-H), 4.64–5.32 (4H, rhamnosyl), 6.17 and 6.33 (3H each, *s*, C-8 and C-7 OC$\underline{H}_3$), 7.67 and 7.86 (3H each, *s*,

* CDRI Communication No. 2289.

phenolic acetates), 7.92, 8.12 and 8.19 (3H each, *s*, aliphatic acetates), 9.24 (*d*, $J = 6$ Hz, 3H). (Found: C, 57.68; H, 4.81. $C_{33}H_{34}O_{16}$ requires: C, 57.72; H, 4.95%).

Hydrolysis of glycoside (**1**). Compound (**1**) 100 mg in 2 ml EtOH on hydrolysis with 6% HCl (20 ml) afforded the aglycone (**2**) mp 262°. Mass: (*m/e*) 330 (M^+). (Found: C, 62.0; H, 4.1. $C_{17}H_{14}O_7$ requires; C, 61.81; H, 4.24%). The aq. acid soln neutralized with (Ag_2O), filtered and evapd to a syrup. PC in BuOH–HOAc–H_2O (4:1:5) indicated the presence of rhamnose. Acetylation of the aglycone **2** yielded the acetate (**3**), mp 200°. NMR ($CDCl_3$): Four A_2B_2 protons of ring B at 2.2 (*d*, $J = 8.5$ Hz, 2H), 2.8 (*d*, $J = 8.5$ Hz, 2H), 2.97 (*s*, 1H), 6.03 and 6.16 (*s*, 6H, OCH_3), 7.55, 7.68 and 7.71 (*s*, 9H, 3 $COCH_3$). (Found: C, 60.37; H, 4.24. $C_{23}H_{20}O_{10}$ requires: C, 60.52; H, 4.38%).

Methylation of the glycoside. Methylation of (**1**) with freshly prepared Ag_2O and MeI afforded a TLC pure methylated product as a viscous mass. This compound was hydrolysed with 6% HCl to give (**4**) mp 108° (EtOH). (Found: C, 63.29; H, 4.95. $C_{19}H_{18}O_7$ requires: C, 63.69; H, 5.02%).

(**1**) was methylated with CH_2N_2 and the product crystallized with MeOH. This methylated product was hydrolysed with 5% HCl for 4 hr. The mixture was then extracted with Et_2O and EtOAc and subsequently washed with H_2O, dried and the solvent removed. The product crystallized from Me_2CO as yellow needles (**5**) mp 205°. It had λ_{max} (MeOH) 272, 326, 380; +$AlCl_3$ 270, 315, 350 and 438 nm. (Found: C, 62.67; H, 4.57. $C_{18}H_{16}O_7$ requires: C, 62.79; H, 4.65%).

Acknowledgements—The authors thank Dr. (Mrs.) Asima Chatterji for providing them with the sample of tambulin. One of the authors (P.K.J.) is also grateful to I.C.M.R. for providing S.R.F.

REFERENCES

1. Chailakhyan, M. Kh. and Lozhnikova, V. N. (1959) *Doklady Adad. Naub. S.S.S.R.* **128**, 1309.
2. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, p. 45. Springer, New York.
3. Horowitz, R. M. (1957) *J. Am. Chem. Soc.* **79**, 6561.
4. Sarin, J. P. S., Singh, S., Garg, H. S., Khanna, N. M. and Dhar, M. M. (1976) *Phytochemistry* **15**, 232.
5. Jurd, L. and Horowitz, R. M. (1957) *J. Org. Chem.* **22**, 1618.
6. Nordstrom, C. G. and Swain, T. (1953) *J. Chem. Soc.* 2764.
7. Chatterjee, A., Malakar, D. and Ganguly, D. (1976) *Indian J. Chem.* **14B**, 233.

Phytochemistry, 1979. Vol. 18, pp. 360–362. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0360 $02.00/0

NOUVEAUX AGLYCONES FLAVONIQUES *O*-METHYLES DERIVES DE LA MEARNSETINE CHEZ *ALLUAUDIA ASCENDENS*

ZAFERA ANTOINE RABESA et BERNARD VOIRIN

Laboratoire de Phytochimie, Département de Biologie Végétale, Université Claude Bernard, Lyon 1, 43 Boulevard du 11 novembre 1918, 69621 Villeurbanne, France

(*Reçu le* 28 *juillet* 1978)

Key Word Index—*Alluaudia ascendens*; Didiereaceae; Centrospermae; 4′-*O*-methylmyricetin; mearnsetin; 3-*O*-methylmearnsetin; 6-methoxymearnsetin; 6-methoxy-3-*O*-methylmearnsetin.

INTRODUCTION

Poursuivant l'étude chimiosystématique des Didiereaceae [1–3], nous avons isolé et identifié chez *Alluaudia ascendens* Drake cinq aglycones flavoniques majeurs; à côté du kaempférol figurent la méarnsétine, et trois flavonols *O*-méthylés, A, B, C, dérivés de celle-ci.

RESULTATS

L'identification de la méarnsétine a été obtenue par diverses données spectrales. En SM, outre la valeur du pic moléculaire (*m/e* 332 (100%)) caractéristique d'une flavone monométhoxylée pentahydroxylée, l'étude de la fragmentation [4] permet d'attribuer deux —OH au noyau A (pic à *m/e* 153 (13%)), un O–Me et deux —OH au noyau B (pic à *m/e* 167 (6%)). Sur le phényle latéral, la localisation des substituants a été assurée grâce aux données de la RMN et de la spectrophotométrie UV-visible: la première révèle en effet la substitution des carbones 3′, 4′, 5′ (*s* 7.30 δ, 2H aromatiques: H-2′ et H-6′), la seconde l'absence de système *o*-diOH ($\Delta\lambda_1$ identiques *in* $AlCl_3$, MeOH neutre et acide); il ne subsiste donc qu'une possibilité structurale: méthoxylation en 4′, hydroxylation en 3′ et 5′. Quant au noyau A, la présence sur le spectre de RMN d'un double doublet à 6.19 δ et 6.38 δ ($J = 2.5$ Hz) caractéristique des protons en 6 et 8 montre classiquement la substitution en 5 et 7. Ces données se révèlent d'ailleurs identiques à celles fournies par la littérature [5–7]; en outre, la comparaison directe des données ultraviolettes et des valeurs de R_f