Pergamon

PII: S0031-9422(97)00738-3

Phytochemistry, Vol. 47, No. 7, pp. 1441–1443, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

FLAVONOID AGLYCONES AND A THIOPHENE DERIVATIVE FROM *HELICHRYSUM CASSIANUM*

ECKHARD WOLLENWEBER,‡ JAN F. STEVENS* and MONIKA IVANCIC†

Institut für Botanik der Technischen Hochschule, Schnittspahnstrasse 3, D-64287 Darmstadt, Germany; * Department of Agricultural Chemistry, † Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, U.S.A.

(*Received in revised form* 27 *June* 1997)

Key Word Index—*Helichrysum cassianum*; Asteraceae-Gnaphalieae; leaf and stem exudate; flavonoid aglycones; α-terthienyl; 2,2′:5′,2″-terthiophene.

Abstract—From the lipophilic exudate found on leaves and stems of *Helichrysum cassianum* six flavonoid aglycones have been identified, including a new natural polymethoxy flavone, 3,5-dihydroxy-6,7,8,4′-tetramethoxyflavone. A further non-polar component was identified as α-terthienyl. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Flavonoid aglycones reported previously for many species of *Helichrysum* include flavones, flavonols, flavanones, and chalcones. Flavones and flavonols with 6- and 6,8-*O*-substitution are of frequent occurrence and *O*-prenylated flavonone and chalcone derivatives are characteristic of the genus [1, 2; see also previous editions]. We have reported exudate flavonoids from *H. aureum*, *H. bracteatum* [3], *H. foetidum* [4] and *H. viscosum* (= *Bracteantha viscosa*; [5]). However, in most other previous papers the localization of the flavonoid aglycones was not recorded. We have now analysed the exudate flavonoids of a *Helichrysum cassianum* cultivar, which is grown for its "everlasting" flowers.

RESULTS AND DISCUSSION

Aerial parts of *H. cassianum* Gaudich., cultivar "Rose Beauty", were rinsed with acetone to dissolve the resinous exudate. After elimination of terpenoids and other lipophilic material, five flavonoids were identified by direct comparison with marker compounds. They were apigenin, scutellarein 6-methyl ether (hispidulin), scutellarein 6,7-dimethyl ether (cirsimaritin), 6-methoxyluteolin (nepetin), and quercetin. A sixth flavonoid aglycone (**1**) appeared as a dark spot with low polarity which turned reddish-brown in UV after spraying with "Naturstoffreagenz A". A small amount of this product was isolated by preparative TLC. The EI mass spectrum showed a molecular ion with *m/z* 374, indicating a flavone or flavonol with two hydroxyl and four methoxyl groups. The appearance of an equally intense $[M-15]^-$ ion further suggested the presence of an 8-methoxy- or possibly 6,8-dimethoxy-substitution. The ^1H NMR spectrum showed two A_2B_2-type aromatic signals (8.20 and 7.16 ppm, *d*, $J = 8$ Hz), four *O*-methyl singlets (3.82, 3.85, 3.91, and 4.02 ppm) and a broad OH-5 singlet at 12.2 ppm. The basic *O*-substitution was thus determined to be 3,5,6,7,8,4′ with a free 5-hydroxy and a 4′-methoxy group. The only other free hydroxyl was located at C-3 by tandem MS equipped with the atmospheric pressure chemical ionization (APCI) source. On collision of the $[MH]^+$ ion (*m/z* 375) with Ar gas molecules, the pseudo-molecular ion gave rise to prominent fragment ions with *m/z* 345 $[MH\text{-}2\ CH_3]^+$, 327 $[MH\text{-}2\ CH_3\text{-}H_2O]^+$, 317 (100%), and 302 $[317\text{-}CH_3]^+$. Loss of water from the $[MH\text{-}2CH_3]^+$ ion, in which the 3-hydroxyl is involved, is stabilized by the adjacent C-4 keto function and the heterocyclic oxygen. The ion with *m/z* 317 is most probably the result of contraction of the C-ring, either by direct loss of O=C=CHOH (or its tautomer O=CH—HC=O, oxalaldehyde) from the pseudo-molecular ion or by loss of CO from the $[MH\text{-}2\ CH]^+$ ion. Loss of neutrals like CO and H_2O on ring contraction was previously observed in FAB–MS–MS spectra of flavonols [6]. Compound **1** was thus identified as 3,5-dihydroxy-6,7,8,4′-tetramethoxyflavone, and proved to be identical with an authentic marker of synthetic origin (compound **7a** in [7]) by co-TLC,

‡ Author to whom correspondence should be addressed.

co-HPLC, UV, ^{1}H NMR and APCL–MS–MS. To the best of our knowledge this is the first time that this flavonol has been found as a natural product.

A further non-polar compound (**2**) appeared as a blue fluorescent spot. It was also isolated by prep. TLC and purified by prep. HPLC. The EI mass spectrum exhibited a very prominent molecular peak at *m/z* 248. The NMR spectrum of **2** showed four proton signals of equal intensity and six carbon signals. To account for the molecular weight, the compound had to be symmetrical with a partial empirical formula $C_{12}H_8$. This leaves a difference of 96 mass units to be attributed to six *O* or three *S* atoms. HR–EI–MS convincingly supported the latter possibility. The molecular ion with *m/z* 248 was in agreement with $C_{12}{}^{32}S_3H_8$ while the isotopic peak at *m/z* 250 (15%) was consistent with $C_{12}{}^{32}S_2{}^{34}SH_8$. Our hypothesis of this product being identical with α-terthienyl (2,2′:5,2″-terthiophene) was confirmed by comparison of our ^{1}H NMR data with those earlier reported for α-terthienyl [8]. In the ^{1}H–^{1}H COSY spectrum, the triplet at 7.11 ppm showed correlations with the doublets at 7.54 ppm and 7.35 ppm. Both doublets interacted with the triplet but not with each other. This allowed assignment of the triplet to H-4/4″. The doublet with $J = 5$ Hz was assigned to H-5/5″ and the upfield doublet with $J = 3.5$ Hz to H-3/3″ by comparison with literature values for thiophene [9]. However, the expected *meta* coupling with $J_{3,5}$ ca 1 Hz was not observed.

α-Terthienyl was first isolated as a natural product from petals [10] and from roots of *Tagetes erecta* [11]. According to [12] it is now known from four tribes of Compositae: Heliantheae, Helenieae, Arctodeae and Cynareae.

EXPERIMENTAL

Helichrysum cassianum was propagated in the private garden of Mr H. Groh. A voucher is kept in the herbarium of E.W. at the Institute of Botany, TH Darmstadt. Air-dried aerial parts were briefly rinsed with acetone to dissolve the exudate material. After evapn of the solvent, the residue was redissolved in a small amount of boiling MeOH, cooled to −18° and centrifuged to eliminate the precipitated fatty constituents. The supernatant was chromatographed over Sephadex LH-20, eluted with MeOH, to separate the phenolic portion from the predominant terpenoid material. Individual flavonoids were identified in relevant fractions by direct TLC comparisons with markers, available in E.W.'s lab, on silica gel and on polyamide (cf [13]). Compounds **1** and **2** were isolated by prep. TLC on silica (toluene : methyl ethyl ketone, 9 : 1).

3,5-*Dihydroxy*-6,7,8,4′-*tetramethoxyflavone* (**1**)

UV data are in full accordance with those reported for this flavonol [7]. For ^{1}H NMR data (600 MHz, DMSO-d_6) see Results and Discussion. HPLC comparison of **1** with a synthetic sample [7] was carried out on a 5 μm LiChrospher RP-18 column (250 × 4 mm) with a linear solvent gradient from 45% to 95% MeOH in 1% aq. HCO_2H over 50 min at 1 ml min^{-1} with detection at 350 nm and gave a RRt to quercetin of 2.16. APCI–MS–MS experiments were performed using the daughter-ion scan mode as before [14] except for the orifice potential and the collision energy which were set at 55 V and 30 V, respectively. The $[MH]^+$ ion with *m/z* 375 yielded fragments with the following *m/z* values (% int.): 345 (77), 327 (63), 317 (100), 302 (55), 299 (20), 135 $[B_2]^+$ (21).

α-*Terthienyl* (**2**)

Final purification was achieved by prep. HPLC on a 10 μm 250 × 10 mm Econosil RP-18 column with MeCN–1% aq. HCO_2H (75 : 25) at 5 ml min^{-1} with detection at 280 nm. Repeated runs yielded a pale yellow solid on rotary evaporation and lyophilization, mp. 89° (lit. 93–94° [15]). UV λ_{max} (MeOH): 349, 250 nm, in agreement with Ref [15]. HR-EIMS (probe) 70 eV, *m/z* (rel. int.): 247.9782 (calc. for $C_{12}{}^{32}S_3H_8$: 247.9788) (100). 249.9739 (calc. for $C_{12}{}^{32}S_2{}^{34}SH_8$: 249.9746) [15]. ^{1}H NMR (600 MHz, DMSO-d_6) δ: 7.11 (2H, *t*, $J = 4$ Hz; H-4 and H-4″), 7.27 (2H, *s*, H-3′ and H-4′), 7.35 (2H, *d*, $J = 3.5$ Hz; H-3 and H-3″), 7.54 (2H, *d*, $J = 5$ Hz; H-5 and H-5″). ^{13}C NMR (150.9 MHz, DMSO-d_6) δ: 124.21 (C-3 and C-3″), 124.84 (C-3′ and C-4′), 125.64 (C-5 and C-5″), 128.39 (C-4 and C-4″), 135.25 (C-2 and C-2″), 135.92 (C-2′ and C-5′). Carbon signals were assigned by comparison with chemical shifts reported for a related thiophene [16].

Acknowledgement—E.W. wishes to thank Mr Helmut Groh for the plant material and Mrs Marion Dörr for technical assistance. We also thank Mr Brian Arbogast (OSU) for recording HR-EI mass spectra and Professor Max L. Deinzer and Professor Victor L. Hsu (OSU) for general encouragement and use of instrumentation. Thanks are further due to Professor Dr T. Horie (The University of Tokushima, Japan) for a sample of synthetic 3,5-diOH-6,7,8,4′-tetramethoxyflavone.

REFERENCES

1. Wollenweber, E., in *The Flavonoids—Advances in Research Since* 1986, ed. J. B. Harborne. Chapman and Hall, London, 1994, pp. 259–335.
2. Bohm, B. A., 1994, ibid.
3. Wollenweber, E., Dörr, M., Fritz, H. and Valant-Vetschera, K. M., *Z. Naturforsch.*, 1997, **52c**, 137.
4. Wollenweber, E., Mann, K., Hochwart, S. and Yatskievych. G., *Phytochem. Bull.*, 1989, **21**, 19.
5. Wollenweber, E., Mann, K., Arriaga-Giner, F.-J., Roitman, J. N. and West, J. G., *Phytochemistry*, 1993, **33**, 871.

6. de Koster, C. G., Heerma, W., Dijkstra, G. and Niemann, G. J., *Biomed. Mass Spectrom.*, 1985, **12**, 569.
7. Horie, T., Kawamura, Y., Yamamoto, H. and Yamashita, K., *Chem. Pharm. Bull.*, 1995, **43**, 2054.
8. Coogan, C. K., Horn, D. H. S. and Lamberton, J. A., *Aust. J. Chem.*, 1965, **18**, 723.
9. Pretsch, E., Simon, W., Siebl, J. and Clerc, T., *Tables of Spectral Data for Structure Determination of Organic Compounds*, 2nd edn. Springer Verlag, Berlin, 1989, p. H265.
10. Zechmeister, L. and Sease, J. W., *J. Am. Chem. Soc.*, 1947, **69**, 273.
11. Uhlenbroek, J. H. and Bijlof, J. D., *Trav. Chim. Pays-Bas*, 1958, **77**, 1004.
12. Sorensen, N. A., in *The Biology and Chemistry of the Compositae*, Vol. 1, ed. V. H. Heywood, J. B. Harborne and B. L. Turner. pp. 400–401.
13. Wollenweber, E., Dörr, M., Beyer, M. and Schilling, E., *Z. Naturforsch.*, 1996, **51c**, 893.
14. Stevens, J. F., Ivancic, M., Hsu, V. L. and Deinzer, M. L., *Phytochemistry*, 1997, **44**, 1575.
15. Merck Index, 11th edn. Merck & Co., Rahway NJ, 1989, p. 9106.
16. Fischer, N. H., Vargas, D. and Menelaou, M., Modern NMR methods in phytochemical studies. in *Modern Phytochemical Methods*, ed. N. H. Fischer *et al.* Plenum Press, New York, pp. 271–317.