Pergamon

0031-9422(95)00924-8

Phytochemistry, Vol. 42, No. 4, pp. 1243–1245, 1996

0031-9422/96 $15.00 + 0.00

GALLOTANNINS AND FLAVONOIDS FROM *HAEMATOXYLON CAMPECHIANUM*

F. E. KANDIL,*† N. H. EL-SAYED,*‡ H. N. MICHEAL,* M. S. ISHAK* and T. J. MABRY†

*National Research Centre, Dokki, Cario, Egypt; †Department of Botany, University of Texas at Austin, Austin TX 78713, U.S.A.

(*Received in revised form* 30 *October* 1995)

Key Word Index—*Haematoxylon compechianum*; Leguminosae; leaves; 2,6-bis-*O*-digalloyl-3-*O*-galloylglucose and 2-*O*-trigalloyl-1,3,4,6-tetrakis-*O*-galloylglucose.

Abstract—Two new compounds, 2,6-bis-*O*-digalloyl-3-*O*-galloylglucose and 2-*O*-trigalloyl-1,3,4,6-tetrakis-*O*-galloylglucose, along with four known gallotannins, were isolated and identified from the leaves of *Haematoxylon campechianum*. In addition, four known flavonoid and three simple phenolic compounds were also detected.

INTRODUCTION

The isolation and structural determination of tannins from various species have provided advances in establishing chemical methods and assays for examining their biological activities [1]. Such activities involved host-mediated anti-tumour activity and anti HIV-activity [2].

Haematoxylon campechianum L., which was collected in Egypt, was previously found to contain neoflavonoids and terpenoids [3]. One of the reported neoflavonoids, haematoxylin, exhibited significant anti-inflammatory activity [4]. The extract of the wood has been used as a source of dyes [5–7].

In our previous investigation [8], we reported the isolation and identification of 18 flavonoid and phenolic compounds from *H. campechianum* twigs. We now report the isolation and identification of two new gallotannins, 2,6-bis-*O*-digalloyl-3-*O*-galloylglucose (**1**) and 2-*O*-trigalloyl-1,3,4,6-tetrakis-*O*-galloylglucose (**2**), in addition to the known gallotannins, 1,2,3,6-tetra-*O*-galloylglucose (**3**), 1,2,3,4,6-penta-*O*-galloylglucose (**4**), 2,6-bis-*O*-digalloyl-1,3-di-galloylglucose (**5**), octa-galloyl glucose (**6**) and the simple phenolics, gallic acid, methyl gallate and ethyl gallate. Also, the known flavonoids, quercetin, kaempferol, Qu 3-*O*-rhamnoside and Qu 3-*O*-glucoside were detected.

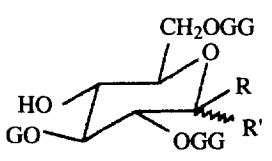
RESULTS AND DISCUSSION

Extraction of *Haematoxylon* leaves with aqueous acetone (80%) eventually gave six known gallotannins and seven known flavonoid components. The structures of the known compounds were confirmed by comparing the results of their chemical degradation, physical and spectral analyses with those reported in literature [9–11].

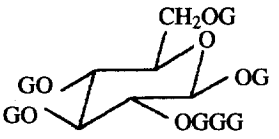
Compound **1** was an off-white amorphous powder which gave, after Sephadex LH-20 and HPLC purification, a dark blue colour with the gallotannin $FeCl_3$ test [12], as well as a positive aniline hydrogen phthalate test for free reducing groups [13]. Acid hydrolysis of **1** gave glucose and gallic acid. The mass spectrum of **1** indicated the compound to be pentagalloyl glucose (m/z 939 by FAB-mass spectrometry), corresponding to a M_r of 940. The ^{1}H NMR spectrum exhibited poorly resolved signals due to the presence of α and β-anomers, together with the *para*- and *meta*-isomers of the depsidically-linked galloyl group, which led to a mixture of all possible isomers.

Based on the better resolved ^{13}C NMR spectrum, compound **1** was assigned the structure 2,6-bis-*O*-di-galloyl-3-*O*-galloyl-(α,β)-D-glucose. The spectrum showed the anomeric C-1 signal of the α-isomer at δ 90.2 with the β-isomer at δ 93.9, the two isomers being present due to the presence of the free anomeric hydroxyl group in the glucose core [14]. The spectrum also showed a downfield shift of the α,β-isomers of C-2 (δ 71.8 and 71.6) and C-6 (δ 63.5 and 61.7) that suggested the presence of more than one galloyl group in both positions, relative to 1,2,3,4,6-pentagalloyl glucose [11]. Signals for (C-3 and C-5) appeared at δ 76.0, while the signal at δ 69.3 is typical of that of a free hydroxyl group at C-4. Compound **1** is therefore a pentagalloyl glucose having two unesterified hydroxyl groups at positions 1 and 4. The two depside galloyl groups (*para-meta*-isomers) at C-2 and C-6 were

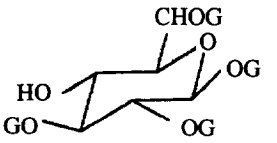
‡Author to whom correspondence should be addressed.



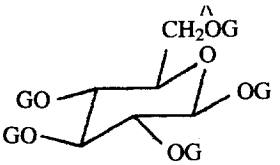
(1)



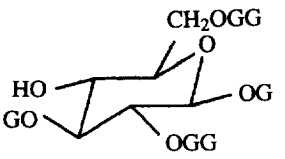
(2)



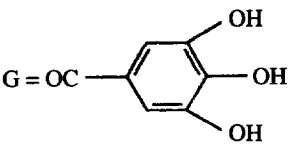
(3)



(4)



(5)



Galloyl

R = OH; R' = H for β isomer

R = H; R' = OH for α isomer

confirmed by the presence of weak signals in the ^{13}C NMR spectrum which are known to be very difficult to resolve [11, 12, 14]. The fifth galloyl group is located at position 3.

This compound can thus be assigned structure **1**, 2,6-bis-*O*-digalloyl-3-*O*-galloyl (α, β) galloyl-D-glucose.

Compound **2** was obtained as a light-brown amorphous powder after Sephadex LH-20 and HPLC purification. It exhibited the same colour reactions as **1**, but gave a negative aniline hydrogen phthalate test, thus indicating the absence of a free reducing group [13]. The negative FAB mass spectrum of **2** showed an ion at *m/z* 1243 $[M-1]^-$ corresponding to a heptagalloylglucose. The ^1H NMR spectrum exhibited a downfield shift of all sugar proton signals, very similar to the pattern observed for 1,2,3,4,6-pentagalloyl glucose. Due to the presence of more galloyl groups attached to the full esterified glucose, a mixture of *para*- and *meta* isomers could be detected from the ^1H NMR spectrum.

The well-resolved ^{13}C NMR spectrum was the key to the structural elucidation of compound **2**. All carbon signals of the glucose core appeared at the same position as in 1,2,3,4,6-pentagalloyl glucose, except for C-2, which showed a slight but significant downfield shift (+0.3 ppm) relative to the equivalent signal for 1,2,3,4,6-pentagalloyl glucose, Together these data established **2** to be 2-*O*-trigalloyl-1,3,4,6-tetrakis-*O*-galloyl glucose [11].

EXPERIMENTAL

Plant material. Leaves of *H. campechianum* L. were collected in July 1991 from the Botanical Garden at Orman and identified by Dr L. Boulos [15]. A voucher specimen is deposited in the Herbarium of the National Research Centre.

Extraction and isolation. Air-dried leaves (2 kg) were extracted with 80% aq. Me_2CO (3 × 2 l) at room temp. for 24 hr, and the solvent removed *in vacuo* to afford 150 g of extract. The concd aq. extract was applied to Sephadex LH-20 column using the solvent system, EtOH (96%), EtOH–H_2O (3:2), EtOh–H_2O–Me_2CO (1:1:2), respectively. Further purification of each fr. was achieved using HPLC on a normal phase column (Develosil 605, (Nomura Chemical, NO2509210) and solvent system 1, *n*-hexane–MeOH–THF–HCO_2H (55:33:11:1) containing 1 g of oxalic acid) [11], flow rate 1.8 ml min^{-1}, UV 280 nm, and a reverse-phase column (RC M8 × 20 Module column (C_{18}), solvent system 2, 0.05 M H_3PO_4–0.05 M KH_2PO_4–EtOH–EtOAc containing 1 g oxalic acid (17:17:4:2), flow rate 2 ml min^{-1}, UV 280 nm [16]. PC (Whatman 2 MM) was carried out using A, 6% HOAC and B, *n*-BuOH–HOAC–H_2O (14:1:5).

2,6-*Bis*-O-*digalloyl*-3-O-*galloyl glucose* (**1**). Off-white amorphous powder. UV λ_{max} 282 nm. (MeOH). R_f v 0.31 (A) 0.52 (B 1:5), R_t (1) 4.6 min. ^{13}C NMR (125 MHz, Me_2CO-d_6, TMS) Glucose moiety: δ 90.8, 93.3 (C-1, B), 71.8, 71.6 (C-2, β), 75.9 (C-3, 5), 69.3 (C-4), 63.5, 61.7 (C-6, β). Galloyl moieties: δ 166.9, 166.8, 166.5, 166.5, 165.8 (*s*, each, carbonyl group signals), 146.0 (C-4), 139.0 (C-3, 5), 120.0–122.0 (C-1), 110 (C-2, 6) 118.0, 116.0 and 114.8 (weak signals for the *p*- and *m*-isomers).

2-O-*Trigalloyl*-1,3,4,6-*tetrakis*-O-*galloyl glucose* (**2**). Light-brown amorphous powder. UV λ_{max} 282 nm (MeOH). R_f 0.39 (A), 0.57 (B). ^1H NMR (360 Mz, Me_2CO-d_6) Glucose moiety: δ 6.35 (1H, *d*, J = 8 Hz, H-1), 6.05 (1H, *t*, H-3), 5.7 (*m*, H-4), 5.66 (*m*, H-

4), 4.6 (*m*, H-5), 4.44 (2H, *m*, H-6). Galloyl moieties: δ 6.98, 6.99, 7.02, 7.05, 7.06, 7.1, 7.2 (*s*, 165.6, 165.3, 165.1, 164.9) (*s*, each, carbonyl group signals), 146 (C-3, 5), 140 (C-4), 119.5–121.0 (C-1) 110.0 (C-2, 6), 114.5, 117.3, 129.9, 132, 133.8, 144, 151 (*s*, each, weak signals for *p*- and *m*-isomers).

Acknowledgements—F. E. K. acknowledges with thanks the Egyptian–U.S.A. Scientific Channel Programme for supporting his PhD studies in the Department of Botany, University of Texas at Austin. This research was also supported by Grants to T. J. M. from the National Institutes of Health (GM-35710) and the Robert A. Welch Foundation (Grant F-130).

REFERENCES

1. Okuda, T., Yoshida, T. and Hatano, T. (1989) *J. Nat. Prod.* **52**, 1.
2. Asanaka, M., Kurimura, R., Koshiura, T., Okuda, T., Mori, M. and Yokoi H. (1988) *Fourth International Conference on Immunopharmacology*, Osaka, Abstract paper, p. 47.
3. Lawlor, G. C. and Martin, S. L. (1959) *J. Soc. Dyers Colourist* **75**, 531.
4. Handa, S. S., Chawla, A. S. and Sharma, A. K. (1992) *Fitotropia* **1**, 15.
5. Paris, R. R. and Rousselet, R. (1958) *Ann. Pharm. Fr.* **16**, 747.
6. Kenji, M., Yutaka, A., Makoto, T., Hiroyaki, K. and Joshinori, S. (1990) Appl. 88/201, 124. 11 Aug. 1988, Jpn. Kokai, Tokyo Koko Jp 02 49 779.
7. Noller, C.R. (1965) *Chemistry of Organic compounds*, 3rd Edn. W. B. Saunder Co., Philadelphia and London.
8. El-Sayed, N. H., Michael, H. N., Kandil, F. E. Ishak, M. S. and Mabry, T. J. (1994) **22**, 763.
9. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.
10. Harborne, J. B. (1988) *The Flavonoids, Advances in Research since 1980.* Chapman & Hall, London.
11. Nishizawa, M. and Yamagishi, T. (1982) *J. Chem. Soc., Perkin Trans 1* 2963.
12. Nishizawa, M. and Yamagishi, T. (1983) *J. Chem. Soc., Perkin Trans 1* 961.
13. Hatano, T., Okonogi, A., Yazaki, K. and Okuda, T. (1990) *Chem. Pharm. Bull.* **38**, 2707.
14. Partridge, J. (1949) *Nature* **164**, 443.
15. Boulos, L. (1983) *Medicinal Plants of North Africa*. Reference Publication, Michigan.
16. Nishizawa, M. Yamagishi, T., Nonaka, G., Nishioka, I. and Ragan, M. A. (1985) *Phytochemistry* **24**, 2411.