PERGAMON

Phytochemistry 51 (1999) 1017–1020

PHYTOCHEMISTRY

Trojanoside H: a cycloartane-type glycoside from the aerial parts of *Astragalus trojanus*

Erdal Bedir[a], Ihsan Çalis[a,*], Rita Aquino[b], Sonia Piacente[b], Cosimo Pizza[b]

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey*
[b]*Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, 84084 Penta di Fisciano, Salerno, Italy*

Received 5 November 1998; received in revised form 4 January 1999; accepted 19 January 1999

Abstract

A novel cycloartane-type glycoside was isolated from the aerial parts of *Astragalus trojanus* along with the known glycosides astragaloside II, astragaloside IV, astragaloside VII, brachyoside B, brachyoside C and the pterocarpan derivative maackiain. The structure of **1** was determined by spectral methods (1-D and 2-D NMR, and FABMS) and established as 3-*O*-β-[α-L-arabinopyranosyl(1 → 2)β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane.

Keywords: *Astragalus trojanus*; Leguminosae; Cycloartane type glycoside; Trojanoside H; Isoflavone; Maackiain

1. Introduction

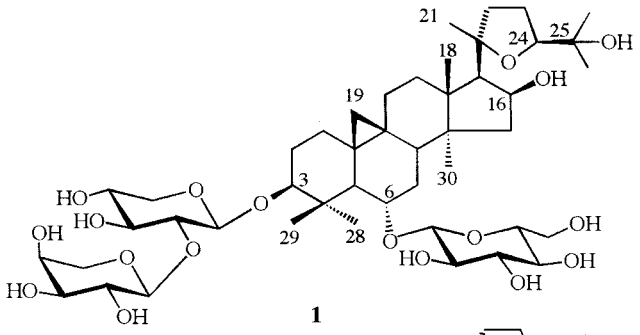
In the flora of Turkey the genus *Astragalus* (Leguminosae) is represented by approximately 380 species which are listed under several sections and are of economical importance for the production of gum (Davis, 1970). Continuing our studies on the constituents of *Astragalus* species (Çalis, Zor, Saracoglu, Isimer, & Rüegger, 1996; Çalis et al., 1997; Bedir, Çalis, Zerbe, & Sticher, 1998a; Bedir, Çalis, Aquino, Piacente, & Pizza, 1998b), we investigated the aerial parts of *A. trojanus*. This paper describes the isolation and structure elucidation of a novel cycloartane-type glycoside, trojanoside H, in addition to the known glycosides astragaloside II (Kitagawa, Wang, Saito, Takagi, & Yoshikawa, 1983), astragaloside IV (Kitagawa et al., 1983a), astragaloside VII (Kitagawa, Wang, & Yoshikawa, 1983), brachyoside B (Bedir et al., 1998b), brachyoside C (Bedir et al., 1998b) and the pterocarpan derivative maackiain (Bedir et al., 1998b).

2. Results and discussion

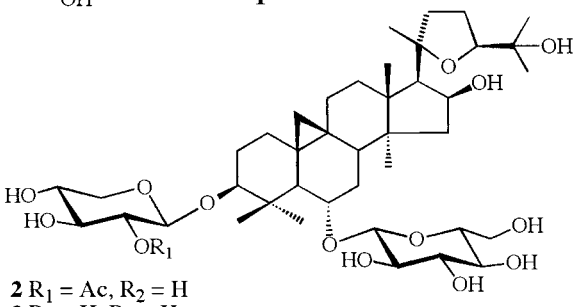
Compound (**1**) ($C_{46}H_{76}O_{18}$) gave a quasimolecular ion peak $[M–H]^-$ at *m/z* 915 and prominent peaks due to the sequential loss of two pentose units $[(M–H)-132]^-$ and $[(M–H)-132x2]^-$, respectively at *m/z* 783 and 651; furthermore a fragment $[(M–H)-162]^-$ at *m/z* 753 due to the loss of a hexose unit from the quasimolecular anion peak was evident. The NMR spectral data of **1** revealed the feature of a cycloartane glycoside (Kitagawa et al., 1983a, 1983b). The ^1H NMR spectra of **1** displayed, for the aglycon moiety, characteristic signals due to cyclopropane–methylene protons as an AX system (δ 0.29 and 0.63, $J_{AX}=4.5$ Hz, H_2-19) and seven tertiary Me groups (δ 1.04, 1.06, 1.16, 1.24, 1.29, 1.30 and 1.32). Additionally the resonance of three anomeric protons, indicative of the presence of three sugar units, were observed in the low-field region at δ_H 4.37 (d, $J=7.8$ Hz), 4.50 (d, $J=7.4$

* Corresponding author. Tel.: +90-312-305-1089; fax: +90-312-311-4777.
E-mail address: acalis@dominet.in.com.tr (I. Çalis)

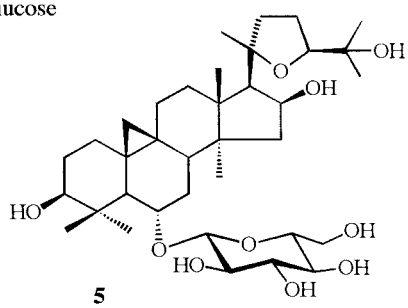
0031-9422/99/$ - see front matter
PII: S0031-9422(99)00035-7



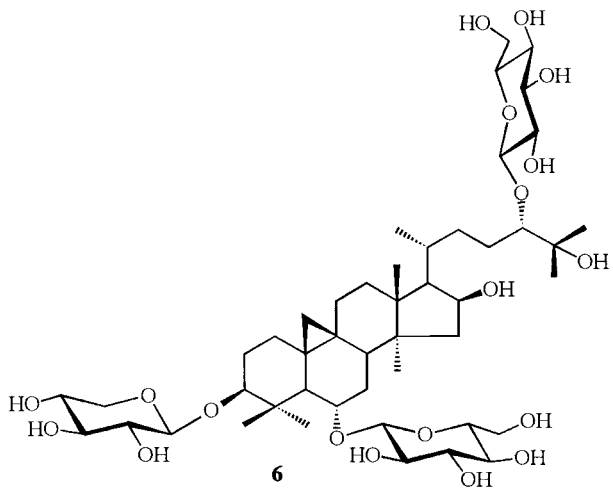
1



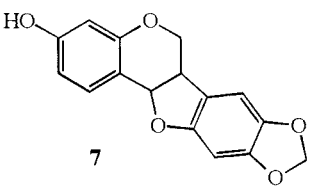
2 R_1 = Ac, R_2 = H
3 R_1 = H, R_2 = H
4 R_1 = H, R_2 = glucose



5



6



7

Hz) and 4.52 (d, $J = 5.8$ Hz). Full assignments of the proton and carbon signals of **1** were secured by ^1H–^1H DQF-COSY and HSQC spectra which allowed the identification of the aglycon of **1** as cycloastragenol [(20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane] glycosylated at C-3 (δ 89.8), C-6 (δ 80.0) and C-24 (82.7) (Kitagawa et al., 1983a, 1983b).

The structure of the oligosaccharide unit was achieved using 1-D-TOCSY (Davis and Bax, 1985) and 2-D NMR experiments. Selected 1-D-TOCSY obtained irradiating each anomeric proton signal yielded subspectra of each sugar residue with high digital resolution. Each subspectrum contained the scalar — coupled protons within each sugar residue. In some cases, because of the small coupling constants, the distribution of magnetization around the spin system was impeded. For this reason, for example, it was possible to identify only three protons (δ 3.82, 3.68, 3.59) coupled to the anomeric signal at δ 4.52 (Table 1). Since in the TOCSY method the cross peaks represent both direct and relayed connectivities, we also recorded a DQF-COSY spectrum (Bodenhausen, Freeman, Morris, Neidermeyer, & Turner, 1977). The results of 1-D-TOCSY and DQF-COSY experiments allowed the sequential assignments of all proton resonances within each sugar residue, starting from the well isolated anomeric proton signals Table 1. Thus on the basis of the chemical shifts, the multiplicity of the signals, the absolute values of the coupling constants, the three sugar residues were identified as L-arabinopyranosyl, β-D-glucopyranosyl and β-D-xylopyranosyl. In the case of the arabinopyranosyl unit the J_{H1-H2} coupling constant (5.8 Hz), midway between that observed for methyl-β-L-arabinopyranoside (4 Hz) and methyl-α-L-arabinopyranoside (8 Hz) has been reported not to be diagnostic on its own, owing to the high conformational mobility of arabinopyranosides ($^4C_1 \rightleftarrows {}^1C_4$). As we reported previously (Piacente, Pizza, De Tommasi, & Mahmood, 1996), evidence of α-L-arabinopyranoside was obtained from a ROESY (Kessler, Griesinger, Kerssebaum, Wagner, & Ernst, 1987) spectrum which showed Nuclear Overhauser effects from C-1ara to C-2ara, C-3ara and C-5ara as expected for an α-L-arabinopyranoside in rapid $^4C_1 \rightleftarrows {}^1C_4$ conformational exchange. HSQC experiments (Bodenhausen and Ruben, 1980) which correlated all proton resonances with those of each corresponding carbon, allowed the assignments of the interglycosidic linkages by comparison of the observed carbon chemical shifts with those of the corresponding methylpyranosides, taking into account the known effects of glycosidation (Breitmaier and Voelter, 1987). The absence of any ^{13}C NMR glycosidation shift for the β-D-glucopyranosyl and α-L-arabinopyranosyl residues suggested these sugars to be terminal. A glycosidation shift was observed for C-2xyl Table 1. The position of the each

Table 1
^{13}C NMR and ^{1}H NMR data of the sugar portion of **1** (CD_3OD, δ ppm, J in Hz, 600 MHz)[a]

Sugar	δ_C	δ_H
Xyl-1 (at C-3 agl)	105.6	4.50 d (7.4)
2	83.2	3.46 dd (7.4, 9.0)
3	76.9	3.55 t (9.0)
4	71.0	3.55 ddd (4.5, 9.0, 11.0)
5	66.0	3.23 t (11.0); 3.88 dd (4.5, 11.0)
Ara 1 (at C-2 xyl)	106.7	4.52 d (5.8)
2	73.5	3.68 dd (5.8, 8.2)
3	74.1	3.59 dd (3.0, 8.2)
4	69.6	3.82 m
5	67.2	3.54 dd (3.0, 12.0); 3.92 dd (2.0, 12.0)
Glc 1 (at C-6 agl)	105.8	4.37 d (7.8)
2	75.7	3.21 dd (7.8, 9.0)
3	78.6	3.36 t (9.0)
4	71.8	3.31 t (9.0)
5	77.8	3.28 ddd (3.0, 4.5, 9.0)
6	62.9	3.68 dd (4.5, 12.0); 3.88 dd (3.0, 12.0)

[a] Assignments confirmed by DQF-COSY, 1-D-TOCSY, HSQC, HMBC.

sugar residues was unambigously determined by the HMBC experiment (Martin and Grouch, 1991) which showed long-range correlations between C-3 (δ 89.8) of the aglycon and H-1xyl (δ 4.50), C-6 (δ 80.0) of the aglycon and H-1glu (δ 4.37), C-2_{xyl} (δ 83.2) and H-1ara (δ 4.52).

On the basis of these evidences, compound **1** was established to be 3-*O*-β-[α-L-arabinopyranosyl-(1 → 2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3β,6α,16β,25-tetrahydroxycycloartane, named trojanoside H.

From the aerial parts of *A. trojanus* astragaloside II (**2**), astragaloside IV (**3**), astragaloside VII (**4**), brachyoside A (**5**), brachyoside B (**6**), the pterocarpan maackiain (**7**) were also isolated and identified by comparison of their ^{1}H and ^{13}C NMR spectral data with literature values (Kitagawa et al., 1983a, 1983b; Wu et al., 1985; Bedir et al., 1998b).

3. Experimental

3.1. General experimental procedures

A Bruker DRX-600 spectrometer operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C using the UXNMR software package was used for NMR measurements in CD_3OD solutions. 2-D experiments: ^{1}H–^{1}H DQF-COSY (Bodenhausen et al., 1977), inverse detected ^{1}H–^{13}C HSQC (Bodenhausen and Ruben, 1980) and HMBC (Martin and Grouch, 1991) and ROESY (Kessler et al., 1987) were obtained by employing the conventional pulse sequences as described previously. The selective excitation spectra, 1-D TOCSY (Davis and Bax, 1985) were acquired using waveform generator-based GAUSS shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5-ms trim pulse. Optical rotations were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm in 0.1% w/v solutions in MeOH. FABMS were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of energy of 2–6 KV).

3.2. Plant material

Astragalus trojanus Stev. (Leguminosae) was collected from Hacibozlar Village, Burhaniye-Balikesir, West Anatolia, in August 1996. Voucher specimens (96–104) have been deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

3.3. Extraction and isolation

The air-dried, powdered roots (390 g) were extracted with 80% EtOH under reflux. The solvent was removed by rotary evaporation, yielding 22.5 g of extract. An aliquot (10 g) of the ethanolic extract was subjected to VLC using reversed-phase material (Sepralyte 40 μm, 100 g) employing H_2O (400 ml), H_2O–MeOH (9:1, 200 ml; 8:2, 200 ml) and MeOH (800 ml). Fractions eluted with MeOH (1.63 g) were rich in saponins. This fraction was further subjected to an open column chromatography (silica gel 60, 75 g) using $CHCl_3$, $CHCl_3$–MeOH (90:10) and $CHCl_3$–MeOH–H_2O mixtures (80:20:1, 80:20:2, 70:30:3 and 61:32:7) yielding twelve fractions (Frs. A–L). Fr. J (90 mg) was applied to a silica gel column (8 g) using $CHCl_3$–MeOH–H_2O (80:20:2) to give **1** (42 mg). Fractionation of fractions A (23 mg), C (42 mg), D (40 mg), F (70 mg), K (75 mg) and L (60 mg) by open column chromatography (silica gel 60; 8 g) using $CHCl_3$, $CHCl_3$–MeOH and $CHCl_3$–MeOH–H_2O mixtures led to the isolation of the other six compounds (**2**–**7**).

3.4. Trojanoside H (*1*)

$[\alpha]_{25}^{D}$ +14.2°; ^{1}H NMR (600 MHz, CD_3OD) Aglycon moiety: δ 4.69 (1H, td, J = 8.0, 5.2 Hz, H-16), 3.80 (1H, dd, J = 8.0, 5.0 Hz, H-24), 3.57 (1H, td, J = 10.0, 4.5 Hz, H-6), 3.23 (1H, dd, J = 11.1, 4.5 Hz, H-3), 2.65 (1H, dd, J = 6.0, 12.0 Hz, H-22a), 2.41 (1H, d, J = 8.0 Hz, H-17), 2.08 (1H, m, H-15a), 2.06 (1H, m, H-23a), 2.02 (1H, m, H-23b), 1.95 (2H, m, H-2a,

H-11a), 1.94 (1H, m, H-7a), 1.92 (1H, m, H-8), 1.71 (2H, m, H-12a, H-2b), 1.69 (1H, m, H-22b), 1.65 (1H, m, H-7b), 1.64 (1H, d, $J=10.0$, H-5), 1.63 (1H, m, H-12b), 1.60 (1H, m, H-1a), 1.43 (1H, m, H-15b), 1.39 (1H, m, H-11b), 1.32 (3H, s, H_3-28), 1.30 (3H, s, H_3-26), 1.29 (1H, m, H-1b), 1.29 (3H, s, H_3-18), 1.24 (3H, s, H_3-21), 1.16 (3H, s, H_3-27), 1.06 (3H, s, H_3-30), 1.04 (3H, s, H_3-29), 0.29 and 0.63 (each 1H, d, $J_{AX}=4.5$ Hz, H_2-19); ^{13}C NMR (600 MHz, CD_3OD) Aglycon moiety: 89.8 (d, C-3), 87.0 (s, C-20), 82.7 (d, C-24), 80.0 (d, C-6), 74.7 (d, C-16), 72.4 (s, C-25), 59.0 (d, C-17), 53.3 (d, C-5), 47.0 (s, C-14), 46.5 (d, C-8), 46.3 (s, C-13), 46.2 (t, C-15), 42.9 (s, C-4), 35.5 (t, C-22), 35.0 (t, C-7), 34.1 (t, C-12), 33.0 (t, C-1), 30.5 (t, C-2), 29.5 (s, C-10), 29.3 (t, C-19), 28.4 (q, C-28), 27.8 (q, C-21), 27.7 (q, C-26), 27.0 (t, C-11), 26.7 (t, C-23), 26.6 (q, C-27), 22.4 (s, C-9), 21.1 (q, C-18), 20.3 (q, C-30), 16.4 (q, C-29); ^{1}H and ^{13}C NMR (600 MHz, CD_3OD) Sugar moiety: Table 1; FABMS m/z 915 $[M-H]^-$, 783 $[(M-H)-132]^-$, 621 $[(M-H)-162]^-$, 601 $[(M-H)-132x2]^-$.

3.5. Astragaloside II (**2**)

^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data superimposable on those reported in the literature (Kitagawa et al., 1983a).

3.6. Astragaloside IV (**3**)

^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data superimposable on those reported in the literature (Kitagawa et al., 1983a).

3.7. Astragaloside VII (**4**)

^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data superimposable on those reported in the literature (Kitagawa et al., 1983b).

3.8. Brachyoside B (**5**)

^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data superimposable on those reported in the literature (Bedir et al., 1998b).

3.9. Brachyoside C (**6**)

^{1}H and ^{13}C NMR (600 MHz, CD_3OD) data superimposable on those reported in the literature (Bedir et al., 1998b).

3.10. Maackiain (7)

^{1}H NMR (600 MHz, CD_3OD) data: δ 7.32 (1H, d, $J=8.5$ Hz, H-1), 6.72 (1H, s, H-7), 6.54 (1H, dd, $J=2.0$ and 8.5 Hz, H-2), 6.44 (1H, s, H-10), 6.42 (1H, d, $J=2.0$ Hz, H-4), 5.89 and 5.92 (each 1H, s, OCH_2O), 5.46 (1H, d, $J=6.0$ Hz, H-11a), 4.21 (1H, dd, $J=5.3$ and 11.4 Hz, H-6eq), 3.64 (1H, t, $J=11.4$ Hz, H-6ax), 3.47 (1H, ddd, $J=5.3$, 6.0 and 11.4 Hz, H-6a); ^{13}C NMR (600 MHz, CD_3OD) data: δ 159.7 (C-3), 157.2 (C-4a), 154.3 (C-10a), 148.2 (C-9), 141.8 (C-8), 132.2 (C-1), 118.0 (C-6b), 112.6 (C-11b), 109.8 (C-2), 104.8 (C-7), 103.8 (C-4), 101.4 (OCH_2O), 93.9 (C-10), 78.6 (C-11a), 66.6 (C-6), 40.2 (C-6a). FABMS m/z 283 $[M-H]^-$.

Acknowledgements

This study was supported by The Scientific and Technical Research Council of Turkey (TUBITAK) (Project no. SBAG 1688). The authors thank Professor Dr. Zeki Aytaç (Gazi University, Faculty of Science, Department of Botany, Etiler, Ankara-Turkey) for the determination of the plant specimen.

References

Bedir, E., Çalis, I., Zerbe, O., & Sticher, O. (1998a) *J. Nat. Prod., 61,* 503–505.

Bedir, E., Çalis, I., Aquino, R., Piacente, S., Pizza, C. (1998b) *J. Nat. Prod., 61,* 1469–1472.

Bodenhausen, G., & Ruben, D. J. (1980). *Chem. Phys. Lett.*, *69*, 185–186.

Bodenhausen, G., Freeman, R., Morris, G. A., Neidermeyer, R., & Turner, J. (1977). *J. Magn. Reson.*, *25*, 559.

Breitmaier, E., & Voelter, W. (1987). In *Carbon-13 NMR spectroscopy* (pp. 380–393). Weinheim, Germany: VCH.

Çalis, I., Yürüker, A., Tasdemir, D., Wright, A. D., Sticher, O., Luo, Y. D., & Pezzuto, J. (1997). *Planta Med.*, *63*, 183–186.

Çalis, I., Zor, M., Saracoglu, I., Isimer, A., & Rüegger, H. (1996). *J. Nat. Prod.*, *59*, 1019–1023.

Davis, P. H. (1970). *Flora of Turkey and East Aegean islands*, *Vol. 4* (pp. 49–254). Edinburgh: University Press.

Davis, D. G., & Bax, A. (1985). *J. Am. Chem. Soc.*, *107*, 7198–7199.

Kessler, H., Griesinger, C., Kerssebaum, R., Wagner, K., & Ernst, R. R. (1987). *J. Am. Chem. Soc.*, *109*, 607–609.

Kitagawa, I., Wang, H. K., Saito, M., Takagi, A., & Yoshikawa, M. (1983a). *Chem. Pharm. Bull.*, *31*, 698–708.

Kitagawa, I., Wang, H. K., & Yoshikawa, M. (1983b). *Chem. Pharm. Bull.*, *31*, 716–722.

Martin, G. E., & Crouch, R. C. (1991). *J. Nat. Prod.*, *54*, 1–70.

Piacente, S., Pizza, C., De Tommasi, N., & Mahmood, N. (1996). *J. Nat. Prod.*, *59*, 565–569.

Wu, L. J., Miyase, T., Ueno, A., Kuroyanagi, M., Noro, T., & Fukushima, S. (1985). *Chem. Pharm. Bull.*, *33*, 3231–3236.