Pergamon

0031-9422(95)00181-6

Phytochemistry, Vol. 40, No. 1, pp. 157–159, 1995

0031 9422/95 $9.50 + 0.00

CYCLOHEXYL BUTENOLIDE GLUCOSIDES FROM EPACRIDACEAE

SØREN DAMTOFT and SØREN ROSENDAL JENSEN

Department of Organic Chemistry, The Technical University of Denmark, DK-2800 Lyngby, Denmark

(*Received* 5 *December* 1994)

Key Word Index—*Trochocarpa laurina*; *Epacris impressa*; Epacridaceae; cyclohexyl butenolide glucosides; trochocarposide; 4-epitrochocarposide.

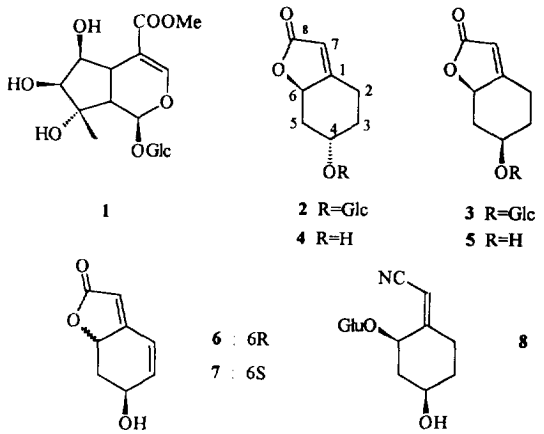
Abstract—*Trochocarpa laurina* contained a new cyclohexyl butenolide glucoside, trochocarposide ((4S,6R)-2,3-dihydro-4-*O*-β-D-glucopyranosyl-aquilegiolide) in addition to the iridoid glucoside schismoside. *Epacris impressa* contained 4-epitrochocarposide ((4R,6R)-2,3-dihydro-4-*O*-β-D-glucopyranosyl-menisdaurilide) which is also a new compound.

INTRODUCTION

Epacridaceae is a medium-sized family of shrubs and small trees mainly from Australia [1]. According to Willis, no feature absolutely distinguishes members of the Epacridaceae from those of Ericaceae, but most botanists keep the two families separate. As part of an ongoing phytochemical investigation of Epacridaceae, we have previously reported the presence of iridoid glucosides in two of its members [2]. The present paper deals with the isolation and structure elucidation of two lactone glucosides of novel structure from the family.

RESULTS AND DISCUSSION

Dry foliage of *Trochocarpa laurina* R. Br. contained, in addition to the earlier reported [2] iridoid glucoside schismoside (**1**), another glucoside of unrelated biosynthetic origin which we have named trochocarposide (**2**). A chemical ionization mass spectrum showed the molecular formula to be $C_{14}H_{20}O_8$ and acetylation gave a tetraacetate (**2a**) with the molecular formula $C_{22}H_{28}O_{12}$, in agreement with the composition of the glucoside. The ^{13}C NMR spectrum of **2** in D_2O showed 14 signals (Table 1) of which six could be assigned to a β-glucopyranosyl moiety. Of the remaining eight signals, three at high field were CH_2-groups while two absorbing at 81.1 and 74.1 ppm pointed to two CHOR groups. Finally, three signals at 112.0, 178.1 and 176.2 ppm, the last two derived from quaternary carbons, indicated the presence of a β,β-disubstituted double bond conjugated with a carboxyl group. Since the aglucone was devoid of free hydroxy groups, and given the molecular formula above, the three low field signals in the ^{13}C NMR spectrum listed above most likely derived from C=CH–COO– in an unsaturated lactone moiety. And finally, a second ring system had to be present. An



1

2 R=Glc
4 R=H

3 R=Glc
5 R=H

6 : 6R
7 : 6S

8

analysis of the ^1H NMR spectrum indicated the structure **2**, in agreement with ^{13}C NMR data given above, and the relevant coupling constants showed that the glucosyloxy- and the lactone groups were *trans*-oriented, giving the relative stereochemistry shown in **2**.

Concurrently, we isolated a similar glucoside **3** from *Epacris impressa* Labill. It had the same gross structure as trochocarposide but in this case the glucosyloxy- and the lactone groups were *cis*-oriented, and we have consequently named the compound epitrochocarposide, with the relative stereochemistry shown.

While both these glucosides were new, the two compounds (4*R*,6*S*) **4** and (4*R*,6*R*) **5** (i.e. aglucones of **2** and **3** or enantiomers of these) have recently been reported from *Sinomenium acutum* Rehder et Wilson (Menispermaceae) [3]. As expected hydrolysis of trochocarposide (**2**) with β-glucosidase gave an aglucone with ^{13}C NMR data identical to those reported for **4** and similarly epitrochocarposide (**3**) gave an aglucone with ^{13}C NMR

Table 1. ^{13}C NMR data

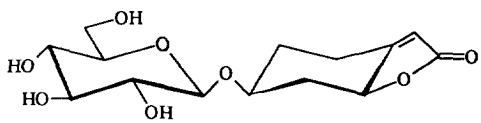
C	**2** D_2O	**2** Pyridine-d_5	**4** Pyridine-d_5	**2a** $CDCl_3$	**3** D_2O	**3** Pyridine-d_5	**5** Pyridine-d_5	**3a** $CDCl_3$
1	176.2	172.6	172.8	—	174.7	171.2	171.8	—
2	23.8	23.5	23.2	23.0	24.1	24.0	24.4	23.7
3	31.8	32.1 (− 1.8)*	33.9	31.7	31.7	32.1 (− 3.5)*	35.6	31.4
4	74.1	72.6 (+ 7.8)	64.8	74.0	75.1	73.5 (+ 7.2)	66.3	74.4
5	37.0	37.8 (− 3.7)	41.5	37.9	40.2	41.4 (− 1.8)	43.2	40.2
6	81.1	78.7	79.2	78.0	81.7	79.8	80.2	79.1
7	112.0	112.4	112.3	112.6	113.7	112.9	112.9	113.5
8	178.1	173.5		—	177.9	173.3	173.6	—
1′	101.4	102.9		100.1	101.8	103.2		99.7
2′	73.8	75.2		71.3	73.7	74.9		71.2
3′	76.5	78.9		72.5	76.4	78.4		72.5
4′	70.5	71.6		68.3	70.3	71.5		68.3
5′	76.7	78.7		71.9	76.7	78.3		71.8
6′	61.5	62.8		61.8	61.5	62.7		61.8

*Glucosidation shifts $\delta_4-\delta_2/\delta_5-\delta_3$.

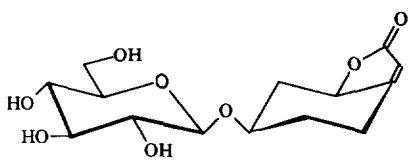
(methanol-d_4) data identical to those of **5**. A C–H COSY experiment of epitrochocarposide, however, showed that the assignments of the high field carbons (C-2, C-3 and C-5) in the reported [3] ^{13}C NMR spectra of the aglucone should be revised as shown in Table 1.

The only remaining questions in the structure elucidation of **2** and **3** were now the absolute configurations. It has been shown [4] that the absolute configuration in a chiral secondary alcohol can be determined using the glycosidation shifts in the ^{13}C NMR spectrum. Thus, in the case of secondary alcohols having two β-CH_2 groups, the glucosidation shift (with a 1-β-D-glucopyranosyl moiety) is *ca* 7.2 ppm for the α-carbon, while −2.2 and −4.0 ppm, respectively, for the β-carbons *syn* and *anti* to the ring-oxygen in the sugar moiety in the most preferred conformation [see refs. 4 and 5] when the spectra are recorded in pyridine-d_6. In Table 1 are also listed the glucosidation shifts calculated for trochocarposide (**2**). Here, $\Delta C\alpha$ was + 7.8 ppm (for C-4) and the $\Delta C\beta$-values were −1.8 and −3.7 ppm (for C-3 and C-5, respectively), which was in excellent agreement with the above figures. This showed that C-3 is *syn* to the glucopyranosyl oxygen and C-5 is *anti* (see Fig. 1). Consequently, the absolute configuration at C-4 in trochocarposide was *S* and it followed that the absolute configuration of C-6 was *R*. The aglucone of trochocarposide (4*S*,6*R* **4**) was thus the enantiomer of the compound reported from *S.acutum* [3]. Similar calculations (Table 1) were made for epitrochocarposide (**3**). In this case $\Delta C\alpha$ was + 7.2 ppm (for C-4) and the $\Delta C\beta$-values were −3.5 and −1.8 ppm (for C-3 and C-5, respectively), showing that C-3 was *anti* to the glucopyranosyl oxygen and C-5 was *syn*, i.e. opposite to the situation above. Consequently, the aglucone of epitrochocarposide (4*R*,6*R* **5**) is identical to the second isomer isolated from *S. acutum* [3].

As stated above, the two cyclohexyl butenolide glucosides are new, but similar compounds are known. In addition to the two aglucones 4*R*,6*S* **4** and 4*R*,6*R* **5**, menisdaurilide (**6**) and aquilegiolide (**7**) together with the



2



3

Fig. 1. Preferred conformations of trochocarposide (**2**) and 4-epitrochocarposide (**3**).

nitrile glucoside menisdaurin (**8**) were also present in *S. acutum* [3]. Menisdaurin had earlier been reported from *Menispermum dauricum* (Menispermaceae) [6] where **6** was obtained as a synthetic product by hydrolysis of **8**. The lactone **7** was first isolated from *Aquilegia atrata* (Ranunculaceae) [7] and later from *Coccolus lauriforius* (Menispermaceae) [8] and *Phyllanthus anisolobus* (Euphorbiaceae) [9], which both also contained the isomer **6**. A number of non-cyanogenic cyanoglucosides are known from plants, but they do not appear to have a significant systematic value since they are widely scattered in the angiosperms [10]. Whether or not the same is the case for the cyclohexyl butenolide glucosides remains to be seen. They were not, however, present in all the investigated members of Epacridaceae (*Astroloma humifusum*, *Leucopogon virgatus* and *L. parviflorus* did not contain any [2]) therefore they cannot be used as a simple distinguishing feature between Epacridaceae and Ericaceae.

EXPERIMENTAL

The plant material was collected in Australia and vouchers were earlier deposited in the herbarium of the Botanic Museum, Copenhagen, Denmark [2]. Prep. chromatography was performed on Merck Lobar reverse phase (C_{18}) columns with H_2O–MeOH mixts as specified. For ^1H NMR, the solvent peaks (δ4.75 in D_2O and δ7.27 in $CDCl_3$) served as standards and in the ^{13}C NMR spectra in D_2O, C-6 was defined as 61.5 ppm [11]. The microanalyses were performed by Preben Hansen, University of Copenhagen, Denmark.

Trochocarpa laurina. Dry plant material (92 g) was blended with EtOH. After 1 week, the mixt. was filtered and evapd. The extract was partitioned between Et_2O and H_2O. The aq. phase was passed through Al_2O_3 which was subsequently eluted with H_2O. Evapn provided a crude extract (3.49 g) which was applied to the Lobar C-column and eluted with H_2O–MeOH (25:1→3:1). Schismoside (**1**) was eluted with 10:1 while elution with 5:1 gave a fraction containing **2** (166 mg). This fraction was rechromatographed on a B-column eluting with 10:1 to give pure trochocarposide **2** (72 mg, 0.08%).

Trochocarposide (**2**). A foam. $[\alpha]_D^{22}$ − 45° (MeOH; *c* 1.3). ^1H NMR (250 MHz, D_2O): δ5.96 (*bs*, H-7), 5.29 (*dd*, *J* = 12 and 6.5 Hz, H-6), 4.47 (*m*, H-4), 2.98–2.90 (2H, *m*, H-2 eq + H-5 eq), 2.79 (*bdt*, *J* = 13, 13 and 5 Hz, H-2ax), 2.33 (*m*, H-3 eq), 1.72 (*ddt*, *J* = 13, 13, 5 and 2 Hz, H-3ax), 1.55 (*dt*, *J* = 13, 13 and 2 Hz, H-5 ax); CI MS *m/z*: 317 $[M + 1]^+$ (10), 155 [aglucone + 1]$^+$ (75), 137 [aglucone − 18 + 1]$^+$ (100) in accordance with gross structure $C_{14}H_{20}O_8$.

Trochocarposide tetraacetate (**2a**). Prepared by acetylation with Ac_2O–pyridine. Crystals from EtOH. Mp 151–156°. Recryst. (EtOH) mp 157–158°. $[\alpha]_D^{20}$ −32.1 (*c* 0.5, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ5.76 (*bs*, H-7), 4.85 (*bdd*, *J* = 12 and 6 Hz, H-6), 4.24 (2H, *m*, 1 × H-6′ + H-4), 2.7 (3H, *m*, 2 × H-2 + H-5 eq), 2.32 (*bd*, *J* = 12 Hz, H-3 eq), 2.1–2.0 (4 × Ac), 1.54 (*m*, H-3 ax), 1.39 (*dt*, *J* = 12, 12 and 2.2 Hz, H-5 ax); ^{13}C NMR: Table 1. (Found: C, 54.4; H, 5.8. $C_{22}H_{28}O_{12}$ required: C, 54.5; H, 5.8%).

Enzymatic hydrolysis of **2**. Trochocarposide (**2**) (2 mg) was dissolved in H_2O (1 ml) and β-glucosidase was added. After 2 days the water was evaporated and the ^{13}C NMR spectra of the crude mixture were recorded. In CD_3OD, signals identical to those reported for (4*R*,6*S*) 2,3-dihydroaquilegiolide (**3**) [3] were seen in addition to signals from α- and β-glucose. Similarly, the aglucone shifts were recorded in pyridine-d_5 (Table 1).

Epacris impressa. Dry plant material (143 g) was blended with EtOH. After 1 week the mixt. was filtered and evapd. The extract was partitioned between Et_2O and H_2O. The aq. phase was passed through Al_2O_3 which was subsequently eluted with H_2O. Evapn provided a crude extract (3.40 g) which was applied to a size C-column and eluted with H_2O–MeOH (10:1 → 7:1). Elution with 10:1 gave crude **3** (110 mg, 0.08%) which was rechromatographed on a B-column to give pure 4-epitrochocarposide (**3**).

4-*Epitrochocarposide* (**3**). A foam, $[\alpha]_D^{20}$ −43.0 (MeOH; *c* 1.4); ^1H NMR (500 MHz, D_2O): δ5.83 (*br t*, *J* = 1.5 Hz, H-7), 4.99 (*ddd*, *J* = 12, 6.5 and 1.5 Hz, H-6), 4.10 (*tt*, *J* = 11.5 and 4 Hz, H-4), 2.90 (*m*, H-2 eq), 2.80 (*m*, H-5 eq), 2.37 (*m*, H-2ax), 2.30 (*m*, H-3 eq), 1.40 (*q*, *J* = 11.5 Hz, H-5ax), 1.37 (*m*, H-3ax); ^{13}C NMR: Table 1; CIMS *m/z*: 155 [aglucone + 1]$^+$ (100), 137 [aglucone − 18 + 1]$^+$ (75) in accordance with gross structure $C_{14}H_{20}O_8$.

4-*Epitrochocarposide tetraacetate* (**3a**). Prepared by acetylation with Ac_2O–pyridine. Amorphous compound, $[\alpha]_D^{20}$ −34.5° ($CHCl_3$; *c* 0.7). ^1H NMR (500 MHz, $CDCl_3$): δ5.78 (*bs*, H-7), 4.73 (*ddd*, *J* = 12, 6 and 1 Hz, H-6), 3.88 (*tt*, *J* = 11.5 and 4 Hz, H-4), 2.89 (*m*, H-2 eq), 2.85 (*m*, H-5 eq), 2.30 (*ddt*, *J* = 6, 1 and 14 Hz, H-2ax), 2.22 (*m*, H-3 eq), 1.45 (*q*, *J* = 11.5 Hz, H-5 ax), 1.42 (*m*, H-3 ax); ^{13}C NMR: Table 1. (Found: C, 54.4; H, 5.8. $C_{22}H_{28}O_{12}$ require: C, 54.5; H, 5.8%).

Enzymatic hydrolysis of (**3**). 4-Epitrochocarposide (**3**, 7 mg) was dissolved in H_2O (1 ml) and β-glucosidase (1 mg) was added. After 3 days the H_2O was evaporated and the ^{13}C NMR spectra were recorded of the reaction mixture. The ^{13}C NMR data in CD_3OD of the aglucone part were identical with those published [3]. Similarly, the spectrum was recorded in pyridine-d_6 (Table 1).

Acknowledgements—We thank Dr M. J. Allenden for substantiation of the plant material, Dr T. Clifford for collection and determination of *Trochocarpa laurina*, National Parks Service (permit 778/25) and Forests Commission (licence 2733) in Victoria for permission to collect plants.

REFERENCES

1. Willis, J. C. (1973) *A Dictionary of the Flowering Plants and Ferns* (8th Ed.) Cambridge University Press, Cambridge.
2. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1993) *Phytochemistry* **33**, 377.
3. Otsuka, H., Ito, A., Fujioka, N., Kawamata, K., Kasai, R., Yamasaki, K. and Satoh, T. (1993) *Phytochemistry* **33**, 389.
4. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100**, 3331.
5. Lemieux, R. U. and Koto, S. (1974) *Tetrahedron* **30**, 1933.
6. Takahashi, K., Matsuzawa, S. and Takani, M. (1978) *Chem. Pharm. Bull.* **26**, 1677.
7. Guerriero, A. and Pietra, F. (1984) *Phytochemistry* **23**, 2394.
8. Yogo, M., Ishiguro, S., Murata, H. and Furukawa, H. (1990) *Chem. Pharm. Bull.* **38**, 225.
9. Bachmann, T. L., Ghia, F. and Torssell, K. B. G. (1993) *Phytochemistry* **33**, 189.
10. Hegnauer, R. (1989) *Chemotaxonomie der Pflanzen* vol VIII p. 67, Birkhäuser.
11. Damtoft, S., Jensen, S. R. and Nielsen, B. J. (1981) *Phytochemistry* **20**, 2717.