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NEW CARDENOLIDES FROM SEEDS OF *CORCHORUS TRILOCULARIS**

D. VENKATA RAO and E. VENKATA RAO

Department of Pharmacy, Andhra University, Waltair, India

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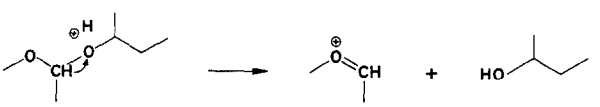
Key Word Index—*Corchorus trilocularis*; Tiliaceae; seed cardenolides; canarigenin 3-*O*-β-D-boivinoside; corchoroside B; structural analysis.

Abstract—From the seeds of *Corchorus trilocularis*, subjected to autofermentation, two crystalline glycosides were isolated. The major glycoside is new and named trilocularin, the minor identical with corchoroside B. Trilocularin was shown to be the 3-*O*-β-D-boivinoside of canarigenin from chemical and spectral evidence.

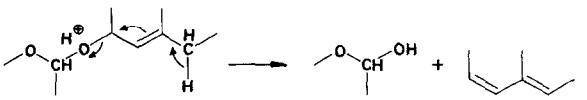
The genus *Corchorus* has been investigated by a number of workers and reported to contain cardiac glycosides [1–9]. Our examination of the seeds of *C. trilocularis* L., which was not investigated earlier, revealed the presence of four cardenolides, A, B, C and D. Of these, compounds A (major) and D (minor) are glycosides. Compound A which is new is named trilocularin, while compound D was found to be identical with corchoroside B [1] whose constitution was reported by us earlier [8].



Scheme 1.

Trilocularin (**1**) crystallized from acetone–ether as fine needles and gave a deep violet colour with the Kedde reaction and an orange–red Legal test showing its cardenolide nature. The elemental analysis ($C_{29}H_{42}O_7$) indicated that it is a glycoside. The UV absorption maxima at 220 nm (log ε 4·2), and IR bands at 1620, 1736, 1775 (α,β-unsaturated γ-lactone) 3400 cm^{-1} (OH group) further confirmed the cardenolide nature of the compound. The presence of an isolated double bond was shown by yellow colour with tetranitromethane. The Keller–Kiliani reaction and xanthydrol tests were positive suggesting that (**1**) contains a 2- or 2,6-dideoxy sugar. Micro Zeisel determination and NMR of (**1**) showed the absence of a methoxyl group but (**1**) formed a diacetate and hence the two acylable hydroxyls are present in the sugar moiety.

Since (**1**) shows a positive Keller–Kiliani reaction it should hydrolyse completely to a sugar and steroid alcohol under very mild conditions [10] according to Scheme 1. Indeed (**1**) hydrolysed under the above conditions to give a sugar and a genin and both were isolated in crystalline form. The genin gave an intense brownish yellow colour with tetranitromethane and did not form an acetate. It was identified as 3,5-dianhydroperiplogenin (**2**) [11]. The UV data (225, 236 nm) also fully agreed with (**2**). Hence, no hydrolysis took place but elimination of the sugar according to Scheme 2. Such a reaction is characteristic of derivatives of substituted allyl alcohols. In cardenolides this phenomenon was first observed in acofrioside L [12] and later in other glycosides derived from canarigenin (**3**), a Δ^4 analogue of digitoxigenin.



Scheme 2.

To secure the genuine aglycone of (**1**) methods described by Meyer *et al.* [11] for a similar glycoside were employed. Hydrolysis of (**1**) with acetone–water–acetic acid proceeded to a large extent with the formation of 3,5-dianhydroperiplogenin (**2**). Chromatography of the hydrolytic product yielded two other crystalline components A and B. Substance A, analysed for $C_{23}H_{32}O_4$ and showed

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a positive tetranitromethane and Rosenheim reactions and formed a monoacetate. These properties are in close agreement with canarigenin (**3**) [11] and the identity was further confirmed by mixed m.p. Substance B also analysed for the formula $C_{23}H_{32}O_4$ and was found to be identical with 3-*epi*-canarigenin (**4**) [11].

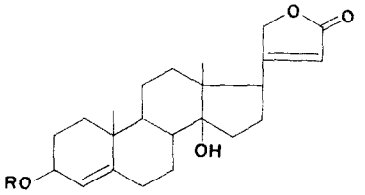
Hydrolysis of (**1**) in the presence of aqueous methanol containing acetic acid also proceeded to a major extent but with little formation of (**2**). Two other minor components of the hydrolytic product were identified as (**3**) and (**4**). The major component (**5**) analysed for $C_{24}H_{34}O_4$ and had one methoxyl. This data and the easy formation of 3,5-dianhydroperiplogenin confirm that (**5**) is 3-*O*-methyl canarigenin (**5**). Since partial epimerization of canarigenin to 3-*epi*-canarigenin occurs in acid solution, the formation of 3,5-dianhydroperiplogenin and the other hydrolytic products suggest that the genuine aglycone in (**1**) could be either canarigenin or *epi*-canarigenin. The proof in support of canarigenin (**3**) was obtained from NMR data. From a study of Dreidung models [11] the angle between the hydrogens at C^{-3} and C^{-4} in canarigenin is about 85° and in 3-*epi*-canarigenin about 35° and therefore the coupling constant should be about 0 cps for canarigenin and about 4 cps for 3-*epi*-canarigenin. 3-*Epi*-canarigenin actually showed a doublet (J 4·5 cps) at δ 5·5 and 3-*O*-acetyl canarigenin showed only a singlet (J 0 cps) at δ 5·27. The NMR spectrum of (**1**) showed an unsplit signal at δ 5·24 due to the vinyl proton at C^{-4} and should therefore be a 3αH-Δ^4 steroid glycoside. A similar conclusion was also reported for canarigenindigitoxoside [11]. It has also been observed [13] that the 6-deoxy group (C^{-5}–Me) in the sugar part appears as a readily identifiable doublet (J 6–7 cps) at δ1·5–1·05. A doublet (J 7 cps) at δ 1·12 in (**1**) confirms the 2,6-dideoxy nature of the sugar.

The MS of (**1**) was very similar to acofrioside L [14] and corchoroside B [8]. The M^+ was absent, as in acofrioside L, and the diagnostically important peaks were at *m/e* 372 (Genin G, $C_{23}H_{32}O_4$ **3**), 354 (G–H_2O, $C_{23}H_{30}O_3$ **2**), 339 (G–H_2O–Me), 336 (G–$2H_2O$), 321 (G–$2H_2O$–Me), 201 ($C_{15}H_{21}$ **9**), 131 ($C_6H_{11}O_3$ 2,6-dideoxy sugar ion).

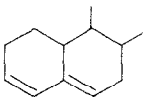
The sugar residue, obtained from the mild acid hydrolysis of (**1**) analysed for $C_6H_{12}O_4$ and corresponded with D-boivinose (**6**) by paper chromatography. However, another 2,6-dideoxy sugar, D-canarose (**7**) has been reported to have the same R_f value but these two sugars can be differentiated by electrophoresis in which (**7**) moves towards the cathode and (**6**) towards the anode [15]. Electrophoresis of the sugar obtained from (**1**) together with authentic canarose and boivinose unequivocally proved the sugar of (**1**) to be D-boivinose.

Application of Hudson's rules can be employed advantageously to determine the α or β configuration of the glycoside and simultaneously to confirm the aglycone as canarigenin. A comparison of the molecular rotations given in Table 1 illustrates this point.

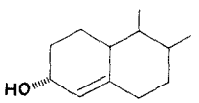
The calculated molecular rotation for canarigenin-β-D-boivinopyranoside is $-24°$ whereas for trilocularin (**1**) it was found to be $-145°$. For canarigenin-α-D-boivinopyranoside the calculated mole-



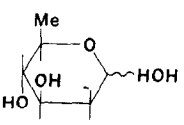
(**1**) (R = β-D-Boivonosyl) trilocularin
(**3**) (R = H) Canarigenin
(**5**) (R = OMe) 3-*O*-Methyl canarigenin
(**8**) (R = α-L-Rhamnosyl) corchoroside B



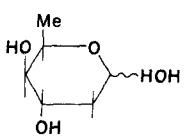
(**2**) 3,5-Dianhydro periplogenin



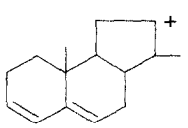
(**4**) 3-*epi*-canarigenin



(**6**) D-Boivinose



(**7**) D-Canarose



(**9**) *m/e* 201 $C_{15}H_{21}$

Table 1. Comparison of molecular rotations of cardenolides

Substance	MW	Rotation	$[M]_D$
Canarigenin	372	Found: +40	+149
β-Methyl D-boivinopyranoside	162	Calc.	−125 [2]
Canarigenin-β-D-boivinopyranoside	502	Calc.	−24
Trilocularin	502	Found: −28·9	−145
α-Methyl D-boivinopyranoside	162	Found: +108	+175 [2]
Canarigenin-α-D-boivinopyranoside	502	Calc.	+257
3-*Epi*-canarigenin	372	Found: +105	+391
3-*Epi*-canarigenin-β-D-boivinopyranoside	502	Calc.	+266
3-*Epi*-canarigenin-α-D-boivinopyranoside	502	Calc.	+566

cular rotation value is highly positive (+257°). Still higher positive values are obtained for boivinosides of 3-*epi*-canarigenin (+566 for α and +266 for β). In spite of the large difference the molecular rotation of trilocularin agrees with canarigenin-β-D-boivinopyranoside (both are negative values) and hence the structure (**1**) proposed for trilocularin is substantiated. This is also in accordance with the Klyne's rule [16] which states that in natural cardiac glycosides D-sugars occur as β-glycosides.

The other two cardenolides isolated in the present work, B and C were identified as 3,5-dianhydroperiplogenin (**2**) and canarigenin (**3**). The presence of these two in minor quantities could be due to the very labile nature of canarigenin glycosides. Both (**2**) and (**3**) are perhaps artefacts.

Working independently and simultaneously on *Digitalis canariensis* Meyer *et al.* [11] and Tschesche *et al.* [17] reported canarigenin digitoxoside. In addition Tschesche *et al.* reported another glycoside, m.p. 192–6° $[\alpha]_D$ − 10° whose structure was given as canarigenin boivinoside. This glycoside was not reported by Meyer and coworkers. From canarigenin boivinoside Tschesche *et al.* did not isolate boivinose and the identity of the sugar was established only by paper chromatographic comparison. Meyer *et al.* also encountered in their work a sugar which had the same R_f value in paper chromatography as that of boivinose, but its rotation was found to be higher than boivinose [18]. This sugar was a hitherto unknown 2,6-dideoxy sugar and named canarose; this sugar was not reported by Tschesche *et al.* Later it was shown by Reichstein *et al.* [15] that boivinose and canarose have the same R_f value in paper chromatography and can be distinguished only by electrophoresis. Meyer *et al.* [19, 20] made further investigations of *D. canariensis* and reported a number of minor glycosides but not canarigenin boivinoside. It was suggested by Meyer that the boivinose of canarigenin boivinoside of Tschesche could be canarose. Thus the true canarigenin boivinoside is for the first time found in *C. trilocularis*. It is probable that trilocularin is only a secondary glycoside and the genuine glycoside with one or more glucose units occurs in nature. The occurrence of canarigenin glycosides in nature is very limited and their distribution in plants is given in Table 2.

Table 2. Distribution of canarigenin glycosides in plants

Name of glycoside	Plant
Acofrioside L (3-*O*-methyl L-rhamnose)	*Acocanthera oppositifolia* [12]
Canarigenin canaroside	*Digitalis canariensis* [11, 19, 20]
Canarigenin digitoxoside	*Digitalis canariensis* [11, 19, 20]
Canarigenin digilanidobioside (not isolated)	*Digitalis canariensis* [11, 19, 20]
Canarigenin D-fucoside	*Digitalis canariensis* [11, 19, 20]
Canarigenin glucosidofucoside	*Digitalis canariensis* [11, 19, 20]
Corchoroside B (L-rhamnose)	*Corchorus capsularis* [8]
	C. trilocularis
Trilocularin (D-boivinose)	*C. trilocularis*

EXPERIMENTAL

Extraction and isolation of cardenolides. The seeds (500 g) of *Corchorus trilocularis* were powdered and extracted with petrol. (4 × 1 litre) at 30°. On concn an oily residue was obtained (60 g). The defatted seed powder was wetted with H_2O (1250 ml), toluene (10 ml) added to cover the H_2O layer and kept in an incubator at 37° for 48 hr. The material was extracted with MeOH (4 × 1·5 l.) until the marc was no longer bitter. The MeOH extract was conc at 45° under red. press. (250 ml) and diluted with MeOH until no more ppt. occurred. It was filtered and the filtrate treated with freshly precipitated lead hydroxide and vigorously shaken for 10 min, and again filtered. The filtrate was adjusted to pH 6 and further conc under red. press. at 45° (250 ml), and left at 0° when a crystalline material deposited (0·6 g (**1**) trilocularin). The mother liquor was fractionated using petrol., Et_2O and $CHCl_3$. The organic layers washed with 2 N Na_2CO_3 soln and H_2O, dried and evaporated (petrol. extract 0·2 g); Et_2O extract 1·012 g; $CHCl_3$ extract 0·2 g). On crystallization from Me_2CO–Et_2O, the Et_2O extract yielded a further quantity of (**1**) (0·5 g). The mother liquor of the Et_2O extract (0·5 g) was chromatographed on alumina (15 g) and eluted with C_6H_6–$CHCl_3$, $CHCl_3$ and $CHCl_3$–MeOH mixtures. $CHCl_3$–C_6H_6 (1:1) and $CHCl_3$ eluates on crystallization furnished 3,5-dianhydroperiplogenin (**2**, 20 mg) and canarigenin (**3**, 20 mg) respectively. $CHCl_3$–MeOH (99:1 and 98:2) eluates on concn yielded a further quantity of (**1**) (208 mg). The $CHCl_3$–MeOH (4:1) eluate gave corchoroside B (**8**, 40 mg). The $CHCl_3$ extract was chromatographed on alumina (7 g) as before and (**1**) (40 mg) and **8** (20 mg) were obtained.

Trilocularin (**1**) crystallized from Me_2CO–Et_2O as fine colourless needles, m.p. 170° (softening) 180–82°/200°/215–16°; $[\alpha]_D^{29}$ − 28·9° (*c* 1·01, $CHCl_3$); (Found: C, 69·5; H, 8·2; $C_{29}H_{42}O_7$ requires C, 69·3; 8·4%). NMR 60 MHz (DMSO, internal standard TMS): δ 0·81 (3H, *s*, Me at C-18), 0·95 (3H, *s*, Me at C-19), 1·12 (3H, *d*, J 7Hz, Me at C-5 of sugar), 2·75 (1H, *s*, α-H at C-17), 4·13 (1H, *s*, β-OH at C-14), 4·52 (1H, *d*, J 6Hz, α-H at C-3), 4·88 (2H, *s*, 2H at C-21), 5·24 (1H, *s*, H at Δ^4), 5·86 (1H, *s*, H at C-22) Kedde reaction, deep violet. Legal reaction, orange–red; Keller–Kiliani test, blue; xanthydrol test, red; Tetranitromethane, pale yellow; Liebermann–Burchard test, rose red–violet–blue–green; 84% H_2SO_4, dark brown–brownish red–violet black; conc. H_2SO_4, dark brown–brownish red–greyish black. *Di-O-acetyl trilocularin*: **1** (100 mg) was treated with C_5H_5N (1 ml) and Ac_2O (0·6 ml) and kept at 20° for 48 hr. Excess reagents were removed and the product was taken up in $CHCl_3$–Et_2O (1:3) (60 ml) and washed with 2 N HCl (10 ml), 2 N Na_2CO_3 (10 ml) and H_2O (10 ml). After drying and evaporation the residue (116 mg) crystallized from Me_2CO–Et_2O as needles, m.p. 234° (sintering) 239–41°; $[\alpha]_D^{29}$ − 5·8°; (*c* 0·92 $CHCl_3$) (Found: C, 67·3; H, 7·7; $C_{33}H_{46}O_9$ requires C, 67·6; H, 7·9%).

Isolation of 3,5-*dianhydroperiplogenin* (**2**): **1** (100 mg) was dissolved in MeOH (10 ml) and 0·1 N H_2SO_4 (10 ml) was added and refluxed for 30 min. MeOH was removed and the aq. suspension was extracted with $CHCl_3$ (the aq. layer was worked up for sugars) and soln evaporated. The residue crystallized as lustrous prismatic plates from Me_2CO–Et_2O, m.p. 208–16°; $[\alpha]_D^{29}$ − 45·3 (*c* 1·1 $CHCl_3$); (Found: C, 77·5; H, 8·4; $C_{23}H_{30}O_3$ requires C, 77·8; H, 8·6%). Kedde reaction, violet; Tetranitromethane, brownish yellow; Keller Kiliani, no blue colour; conc. H_2SO_4, carmine red, deep lilac to violet.

Identification of D-*boivinose* (**6**): The aq. acidic soln left after extraction with $CHCl_3$ from the hydrolysis was neutralized with freshly precipitated $BaCO_3$, filtered and evaporated to a syrupy residue. It crystallized from Et_2O–Me_2CO as thick prisms, m.p. 97–100°; $[\alpha]_D^{29}$ − 13·8° (*c* 0·72 Me_2CO) (Found: C, 48·8; H, 8·5; $C_6H_{12}O_4$ requires C, 48·6; H, 8·2%). Keller–Kiliani reaction, blue; xanthydrol reaction, red; m.m.p. with authentic D-boivinose was undepressed. PC (Whatman No. 1) was carried out in the following systems: toluene–BuOH (1:1)/H_2O for 20 hr; toluene–BuOH (1:2)/H_2O for 16 hr; toluene–methyl ethyl ketone (1:1)/H_2O for 48 hr. The papers were developed with vanillin–perchloric acid reagent. Paper electrophoresis was carried out on Whatman No. 4 using borate buffer (pH 10·4) as solvent for 3·5 hr at 1200 V and 45–50 mA. The papers were developed with vanillin–perchloric acid reagent.

Isolation of canarigenin (**3**): **1** (250 mg) was dissolved in Me_2CO (25 ml) and H_2O (25 ml) and HOAC (0·5 ml) were added and kept at 37° for 10 days. The reaction mixture was neutralized with 2N Na_2CO_3, the Me_2CO removed and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 2 N Na_2CO_3 and H_2O, dried and evaporated. The residue (180 mg) showed on TLC. (silica gel, $CHCl_3$–MeOH, 9:1) four spots corresponding with the original glycoside, 3,5-dianhydroperiplogenin, canarigenin and 3-*epi*-canarigenin. It was then chromatographed on alumina (7·5 g) and eluted with C_6H_6, $CHCl_3$ mixtures and $CHCl_3$. The C_6H_6–$CHCl_3$ (95:5) eluate crystallized as white shining plates, m.p. 210–15° (27 mg). M.m.p. with authentic 3,5-dianhydroperiplogenin was undepressed. The C_6H_6–$CHCl_3$ (1:1) eluate on concn and crystallization gave clusters of crystals from Me_2CO–Et_2O (30 mg), m.p. 209–12°; $[\alpha]_D^{30}$ + 105° (*c* 0·62 $CHCl_3$) (Found: C, 74·5; H, 8·4; $C_{23}H_{32}O_4$ requires C, 74·2; H, 8·7%). It was identified as 3-*epi*-canarigenin. The $CHCl_3$ eluate yielded prismatic plates on crystallization from Me_2CO–Et_2O, (15 mg) m.p. 214–22°; $[\alpha]_D^{30}$ + 40° (*c* 0·69 $CHCl_3$) (Found: C, 74·5; H, 8·3; $C_{23}H_{32}O_4$ requires C, 74·2; H, 8·7%). M.m.p. with authentic canarigenin was undepressed. Canarigenin (20 mg) was acetylated with C_5H_5N (0·2 ml) and Ac_2O (0·1 ml). The acetate crystallized as long needles, m.p. 212–15°; $[\alpha]_D^{29}$ − 4·8° (*c* 0·52 $CHCl_3$) (Found: C, 72·6; H, 8·6%; $C_{25}H_{34}O_5$ requires C, 72·4; H, 8·3%).

Isolation of 3-*O-methyl canarigenin*: **1** (200 mg) was dissolved in MeOH (20 ml) and H_2O (20 ml) and HOAC (0·4 ml) were added and the mixture kept at 37° for 10 days. MeOH was removed and the reaction mixture extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 2 N Na_2CO_3 soln, H_2O and then dried and evaporated. The residue (160 mg) which showed five spots on TLC (silica gel, $CHCl_3$–MeOH 9:1) was chromatographed on alumina (7·5 g). The C_6H_6, $CHCl_3$ (3:2 and 1:1) eluates on concentration and crystallization from Me_2CO gave thick prisms, m.p. 212–20°; $[\alpha]_D^{29}$ + 27·1° (*c* 0·501 $CHCl_3$); (Found: C, 74·4; H, 8·5; OMe, 7·6; $C_{24}H_{34}O_4$ requires C, 74·6; H, 8·9; OMe (1), 8·0%). It was identified as 3-*O*-methyl canarigenin.

Corchoriside B (**8**) crystallized from MeOH as prisms, m.p. 210/240–43°; $[\alpha]_D^{28}$ + 60° (*c* 0·95 MeOH) (Found: C, 67·3; H, 8·3; $C_{29}H_{42}O_8$ requires C, 67·2; H, 8·2%). Kedde reaction, violet; Keller–Kiliani reaction, no blue colour; Tetranitromethane, pale yellow; Liebermann–Burchard test, rose red–violet–blue–green; 84% H_2SO_4 purple–violet–grey; conc. H_2SO_4, brownish red–purple–violet–grey. Mixed m.p. with authentic corchoroside B was undepressed. **8** (200 mg) was acetylated as described under trilocularin and the product crystallized from Me_2CO–Et_2O as plates, m.p. 170–74°/84° (Found: C, 64·9; H, 7·7; $C_{35}H_{48}O_{11}$ requires C, 65·2; H, 7·5%).

Micro Kiliani hydrolysis of **8**: **8** (5 mg) was treated with Kiliani mixture and heated at 100° for 1 hr. The solvents were removed and H_2O (1 ml) added and extracted with $CHCl_3$. The aq. layer was passed through an ion exchange resin (Dowex 2, carbonate form) and evaporated. The residue was examined for sugars by PC and found to contain only L-rhamnose.

Isolation of 3,5-*dianhydroperiplogenin and* L-*rhamnose*: **8** (100 mg) was hydrolysed as described under trilocularin. The agly-

cone crystallized from Me_2CO–Et_2O as glistening plates, m.p. 208–14°. M.m.p. with 3,5-dianhydroperiplogenin was undepressed. The sugar moiety was isolated as described under trilocularin. The residue crystallized from Me_2CO–Et_2O as prisms, m.p. 73–85°; $[\alpha]_D$ + 9·2° (c 0·82 H_2O). PC and TLC with authentic L-rhamnose proved the identity of the sugar as L-rhamnose.

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