DULCITOL AND (−)-4'-*O*-METHYLEPIGALLOCATECHIN FROM *KOKOONA ZEYLANICA*[1]

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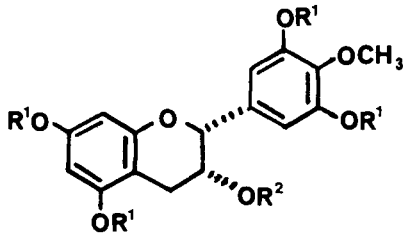
ABSTRACT.—A phytochemical investigation of the methanolic extract of the inner root bark of *Kokoona zeylanica* Thwaites (Celastraceae) has resulted in the isolation and identification of dulcitol and (−)-4'-*O*-methylepigallocatechin. The chemotaxonomic significance and biological activity of dulcitol are discussed.

Kokoona zeylanica Thwaites (Celastraceae), a plant having restricted distribution in Sri Lanka and South India, has an outer bark with a brilliant yellow color (1). According to Thwaites (2), the powdered yellow bark is used as a cephalic snuff to relieve headaches. A paste prepared by mixing powdered bark with water is dried and formed into flat pieces for use by the villagers in Sri Lanka in place of toilet soap (3). The genus *Kokoona* has eight species (2,4), all distributed in the Asian subcontinent.

Our previous work on the stem bark of *K. zeylanica* resulted in the isolation and characterization of friedelin, D:A-friedo-oleanan-3,21-dione, 21α-hydroxy-D:A-friedo-olean-3-one, eight new D:A-friedo-oleanane triterpenes (5–7) and zeylasterone, the first natural phenolic 24-nor-D:A-friedo-oleanane (8). In continuing our studies, we have investigated the methanolic extract of the inner root bark of *K. zeylanica*. In this paper we present the isolation and identification of dulcitol and (−)-4'-*O*-methylepigallocatechin (1). A preliminary report of this work has already appeared (9). Our studies on the minor constituents of this extract will be reported in a subsequent paper.



(**1**), $R^1=R^2=H$
(**2**), $R^1=R^2=COCH_3$
(**3**), $R^1=CH_3$; $R^2=H$

EXPERIMENTAL[4]

PLANT MATERIAL.—The root bark of *Kokoona zeylanica* was collected in the Kanneliya

[1]Part 5 in the series, 'Studies on Medicinal and Related Plants of Sri Lanka.' Part 4 is S. N. Arseculeratne, A. A. L. Gunatilaka and R. G. Panabokke, *J. Ethnopharmacology* **4**, 159 (1981).

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[4]Melting points were taken on a Kofler hot stage apparatus and are uncorrected. The ir spectra were determined on a Perkin-Elmer model 257 recording spectrometer in KBr pellets. The nmr spectra were recorded at 60 MHz on a Varian T60-A spectrometer with tetramethylsilane as internal standard and chemical shifts recorded in δ (ppm) units. The mass spectra were taken on an AEIMS-9 mass spectrometer. Dulcitol for comparison was obtained from B.D.H., England.

rain forest. The identification was verified by Prof. S. Balasubramaniam, Department of Botany, University of Peradeniya, and a herbarium specimen is on file in the University of Peradeniya.

One kg of the dried and ground inner root bark[5] was successively and exhaustively extracted with hot light petroleum ether, chloroform and methanol. Concentration of the hot methanolic extract gave 2.73 g of a pink-colored amorphous solid. The filtrate, when evaporated, yielded 40.4 g of a brown residue, which was extracted with hot ethyl acetate. Evaporation of the ethyl acetate extract afforded 7.1 g of a dark brown solid.

ISOLATION OF DULCITOL.—The above amorphous solid (2.73 g) separated on concentration of the methanolic extract was acetylated by heating for 3h at 100° with acetic anhydride (3 ml) and pyridine (10 ml). The usual work up and recrystallization from methanol gave dulcitol hexa-acetate as a white crystalline solid, mp 162–164° [lit. mp 168° (10)]; ms *m/e* 434 (M^+, 0.3%), 375 (11%), 361 (15%), 289 (43%), 259 (35%), 217 (54%), 187 (69%), 170 (35%), 157 (46%), 145 (61%), 139 (43%), 128 (37%), 127 (30%), 115 (100%), 103 (43%). Deacetylation of the above hexa-acetate with sodium methoxide afforded dulcitol (2.43 g, 0.243%) as a white crystalline solid, mp and mixed mp 187–189° [lit. mp 188° (10)].

ISOLATION OF (−)-4'-*O*-METHYLEPIGALLOCATECHIN (**1**).—The above ethyl acetate extract (7.10 g) was chromatographed over a silica gel column made up in petroleum ether and eluted with a solvent gradient of petroleum ether and ethyl acetate. Elution with 50% ethyl acetate in petroleum ether afforded the major compound (0.0415 g, 0.041%), which was identified as (−)-4'-*O*-methylepigallocatechin (**1**) by the following evidence: mp 140–142°, $[\alpha]_D$ −55° (acetone) [lit. mp 142–144°, [6] $[\alpha]_D$ −53° (11)]; ν_{max} (KBr disc) 3660–3600 (broad), 1660 and 1600 cm^{-1}; pmr (D^6-acetone) δ 7.86 (4H, broad s, exchangeable with D_2O), 6.73 (2H, s, Ar*H*), 6.00 (2H, dd, J=6 Hz and 2 Hz, Ar*H*), 4.90 (1H, d, J=1 Hz, O-C*H*-Ar), 4.26 (1H, m, -C*H*-OH); 3.86 (3H, s, OCH_3), and 2.86 (2H, d, J=4 Hz, $ArCH_2$-).

3,5,7,3',5'-PENTA-*O*-ACETYL-4'-*O*-METHYLEPIGALLOCATECHIN (**2**).—This was obtained as a non-crystallizable amorphous solid by treatment of **1** with acetic anhydride in pyridine at room temperature. It had the following pmr spectral properties supporting the structure **2**; pmr ($CDCl_3$) δ 7.00 (2H, s, ArH), 6.56 (2H, dd, J=6 Hz, 2 Hz, ArH), 5.30 (1H, m, -C*H*OAc), 4.96 (1H, br. s, O-C*H*Ar), 3.73 (3H, s, OCH_3), 2.80 (2H, d, J=4 Hz, $ArCH_2$), 2.20 (6H, s, $-OCOCH_3$), 2.13 (6H, s, $OCOCH_3$), 1.80 (3H, s, $OCOCH_3$).

5,7,3',4',5'-PENTA-*O*-METHYLEPIGALLOCATECHIN (**3**).—Treatment of **1** with excess of diazomethane in ether at room temperature afforded **3** as a white crystalline solid; mp 162–163° [lit., mp 160–162° (11)]; ν_{max} (KBr disc) 1625 and 1590 cm^{-1}; pmr ($CDCl_3$) δ 6.75 (2H, s, Ar*H*), 6.16 (2H, dd, J=6 Hz, 2 Hz, Ar*H*), 4.93 (1H, br s, OC*H*Ar), 4.30 (1H, m, -C*H*OH), 3.90 (6H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 2.91 (2H, d, J=4 Hz, $ArCH_2$-); ms, *m/e* 376 (M^+, 65%), 358 (29%), 211 (38%), 210 (75%), 209 (44%), 195 (75%), 182 (49%), 181 (67%), 168 (58%), 167 (100%), 151 (38%), 138 (38%), 137 (54%), 135 (38%), 121 (37%), 109 (48%), 107 (38%), 105 (32%), 97 (30%), 95 (41%), 91 (41%), 85 (60%), 83 (70%) and 77 (44%).

RESULTS AND DISCUSSION

The powdered plant material was subjected to successive extraction with hot light petroleum ether, chloroform and methanol. The hot methanolic extract, on concentration, yielded a solid which was constituted mainly of dulcitol. Purification via hexa-acetate afforded dulcitol. Identity of the compound was established by comparison with an authentic sample. The mother liquor obtained after the removal of dulcitol was evaporated and extracted with hot ethyl acetate. Column chromatographic purification of this product afforded the major constituent as a colorless amorphous solid, $C_{16}H_{16}O_7$, mp. 140–142°. On acetylation it gave an amorphous pentaacetate, whereas methylation resulted in tetra-*O*-methyl ether. The physical data and spectral properties of the parent compound and these derivatives (see Experimental section) indicated the natural product to be (−)-4'-*O*-methylepigallocatechin (**1**). Comparison of the tetra-*O*-methyl ether with an authentic sample of **3** (11) confirmed its identity.

The presence of dulcitol in *K. zeylanica* is of chemotaxonomic significance since it is a hexitol previously found only in Celastraceae (12, 13) and some genera of Scrophulariaceae (14). Dulcitol has been shown to inhibit the PS leukemia at a dose of 500 mg/kg but was not cytotoxic (13). It may be of interest to note that dibromodulcitol is currently being clinically evaluated as an antitumor agent in humans (15).

(−)-4'-*O*-Methylepigallocatechin (**1**) is an unusual natural catechin that has

[5]Two layers of bark can be recognized in the mature root. The outer layer is brilliant yellow, the inner is colorless, and they can be separated from each other.

been encountered previously only twice, in both instances in plants belonging to Celastraceae (11, 16). Therefore, the occurrence of **1** in *K. zeylanica* is also of chemotaxonomic significance.

ACKNOWLEDGMENTS

The authors thank the United States Department of Agriculture (USA) and the International Foundation for Science (Sweden) for financial support. Dr. G. B. Marini-Bettolo kindly provided an authentic sample of **2**. Messrs. P. H. S. S. A de Silva, P. Liyanage and D. V. Ariyapala are thanked for technical assistance. We thank Mrs. S. C. Weerasekera for typing the manuscript.

Received 26 May 1981

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