analysis of their permethylated derivatives [5]. Exhaustive methylation of the saponin by Hakomori's method [6] gave the deca-*O*-methylate which showed $^1$H NMR signals for ten *O*-methyls (δ3.23–3.94), three anomeric protons (4.20, 1H, $J = 5$ Hz; 4.40, 1H, $J = 6$ Hz; 4.47, 1H, $J = 6$ Hz) and an olefinic proton (5.43, 1H). Hydrolysis of the permethylated saponin gave oleanolic acid, as the aglycone portion, and not the methyl ester suggesting an attachment of a sugar unit at the carboxyl end in the original saponin. GLC analysis of the sugar portion indicated the presence of two sugars in the ratio of 2:1 which were identified as 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-L-arabinose (PC and co-PC in three solvent systems and comparison of $R_g$ values) [7, 8]. Partial hydrolysis of the saponin with 5 N $NH_4OH$ for 1 hr [9] and examination of the sugar portion showed the presence of glucose only.

From the above results it was concluded that a disaccharide, 4-(D-glucopyranosyl)-L-arabinose, was attached via the anomeric hydroxyl of arabinose to the C-3 of the aglycone and also that a molecule of glucose was involved in an ester linkage with the –COOH of oleanolic acid. The observation that the saponin was non-reducing and that hydrolysis of the permethylated saponin gave 2 mol of identically methylated glucose molecules confirmed the involvement of the anomeric hydroxyl of glucose in the ester link with the –COOH group.

Information concerning the pyranose form of the sugars and the configuration of the glycosidic linkages was obtained from the coupling constants of the anomeric protons [10] in the $^1$H NMR spectrum of the permethylated saponin. This was further supported by molecular rotation measurements [9, 11, 12]. The molecular rotation of the saponin $[M]_D$ was observed to be 182.4° showing a difference of 37° from the calculated value of 219.3°. The structure of the saponin was, thus determined to be 3-*O*-[β-D-glucopyranosyl-(1 → 4]-α-L-arabinopyranosyl]-oleanolic acid-(28 → 1)-β-D-glucopyranosyl ester **1**.

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# ERIOSIDE, A NEW COUMARIN GLUCOSIDE FROM *LASIOSIPHON ERIOCEPHALUS**

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**Key Word Index**—*Lasiosiphon eriocephalus*; Thymelaeaceae; 6,8-dihydroxy-7-*O*-β-D-glucosyloxycoumarin.

*Lasiosiphon eriocephalus* Decen. (Thymelaeaceae) is a small tree or branched bush commonly distributed throughout the Western Ghats and Niligiri [1, 2]. The genus comprises of *ca* 25 species, all endemic to tropical Africa; *L. eriocephalus* is the only species found in India. The genus is reputed for its medicinal and toxic properties [3]. The glycosidic extract of *L. kraussianus* is useful as an antileprosy medicament [4, 5]. Mezerein, a phorbol diterpene ester isolated from *L. bruchelli* [6], has been shown to possess antileukemic activity. The Thymelaeaceae has been found to be rich in bicoumarins and two members of this group, lasiocephalin [7] and lasioerin [8], have been isolated from *L. eriocephalus*. The present studies did not reveal any constituent belonging to the phorbol diterpene ester group in this plant but yielded a

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number of phenolic constituents belonging to coumarin, flavonoid and lignan groups. The isolation and structure elucidation of a new coumarin glucoside, erioside, is described.

Erioside, $C_{15}H_{16}O_{10}$, developed an intense yellow colour with alkali, fluoresced white in UV light and gave a positive Fiegel test indicating that it was a coumarin glycoside. On hydrolysis with β-D-glucosidase, it yielded glucose and an aglycone which could not be isolated as such because of its unstable nature. Erioside yielded a hexaacetate, $C_{27}H_{28}O_{16}$ ($M^+$ 608), which showed the presence of two phenolic OH groups in the molecule (see below).

Permethylation of erioside by Hakomori [9] method (DMSO–NaH–MeI) resulted in a complex mixture but its methylation with DMS followed by acid hydrolysis yielded the aglycone (eriosin) dimethyl ether. The positions of the two OMe's in eriosin dimethyl ether have been established on the basis of NOE. The irradiation of the methoxyl signal at δ 3.81 caused a 20% increase in the integrated intensity of the C-5 proton at 6.22, while the C-4 proton at 7.84 was unaffected. The saturation of the methoxyl signal at 3.80 did not cause any change in the spectrum. This established that the functionalities in the molecule (2 OMe, OH) were located at C-6, C-7 and C-8 only.

On methylation eriosin dimethyl ether yielded a trimethoxycoumarin identical to 6,7,8-trimethoxycoumarin, and eriosin dimethyl ether was found to be identical with isofraxidin (mp, IR, NMR). Thus, the structure of erioside was confirmed as 6,8-dihydroxy-7-O-β-D-glucosyloxycoumarin.

## EXPERIMENTAL

The reported mps are uncorr. The $^1$H NMR spectra were recorded in $CDCl_3$, unless stated otherwise, with TMS as int. standard. The NOE experiment was performed on a Perkin–Elmer R-32 NMR spectrometer and the adjustment of irradiation frequency at δ 3.80 or 3.81 was made by frequency offset knob (each division = δ 0.01). $R_f$ values are related to Si gel plates using ceric sulphate as spray reagent.

The plant material was collected from Mahabaleshwar (Maharashtra, India) and identified by Dr. B. M. Mehrotra. A voucher specimen NO. 684 is preserved in the herbarium of the Institute.

The EtOH extract of the dried plant material (3 kg) was fractionated into $Et_2O$, $CHCl_3$, EtOAc and *n*-BuOH fractions. The EtOAc extract (25 g) was chromatographed over Si gel (1.5 kg) and 40 fractions (500 ml each) were collected using $CHCl_3$–MeOH–$H_2O$ (35:9:2). Fractions 21–25 (1.012 g) on crystallization with MeOH yielded substance H (196 mg).

*Substance H (erioside).* Pale yellow needles, mp 350° dec., $R_f$ 0.5 ($CHCl_3$–MeOH–$H_2O$, 35:9:2), $\nu_{max}^{KBr}$ cm$^{-1}$: 3350 (OH), 1680 (α-pyrone), 1624 (chelated CO), 1590, 1518 (Ar), 1280 (Ar–O–C=C–), 1260, 1242, 1200, 1128, 1078, 1038, 1020, 860, 840. $\lambda_{max}^{MeOH}$ nm: 267, 329 (log ε 3.66, 3.95). $^1$H NMR (DMSO-$d_6$): δ 3.95 (3H, *m*, C-5', C-6'), 4.4 (4H, *m*, C-1', C-2', C-3', C-4'), 5.68 (1H, *d*, *J* = 10 Hz, C-3), 5.98 (1H, *s*, C-5), 7.76 (1H, *d*, *J* = 10 Hz, C-4). (Found: C, 50.58; H, 4.50. $C_{15}H_{16}O_{10}$ requires: C, 50.56; H, 4.49%.) Erioside ($Ac_2O$–$C_5H_5N$ overnight at room temp.) yielded peracetate (MeOH), mp 180°. $\nu_{max}^{KBr}$ cm$^{-1}$: 1740 (Ar–OAc). $^1$H NMR: δ 2.01 (9H, *s*, 3 OCOMe), 2.18 (3H, *s*, OCOMe), 2.3 and 2.36 (3H each, *s*, ArOCOMe), 3.55–4.45 (3H, *m*, C-5', C-6'), 5.25 (4H, *m*, C-1', C-2', C-3', C-4'), 6.33 (1H, *d*, *J* = 10 Hz, C-3), 6.9 (1H, *s*, C-5), 7.63 (1H, *d*, *J* = 10 Hz, C-4). MS *m/e* (rel. int. %): 608 (1.6), 331 (35.39), 278 (3.02), 277 (0.48), 271 (5.55), 236 (8.70), 235 (2.0), 229 (4.0), 211 (4.18), 194 (32.08), 169 (100), 166 (2.13), 145 (9.2), 139 (10.13), 138 (1.02), 127 (32.18), 109 (100). Erioside (10 mg) and β-glucosidase (5 mg) dissolved in acetate buffer (pH 5) (2 ml) were allowed to stand overnight at 37°. The solution was concd *in vacuo* and extracted with EtOAc. PC of the aq. phase (BuOH–$C_5H_5N$–$H_2O$, 6:4:3) indicated one spot due to glucose, which was confirmed by co-PC in BuOH–HOAc–$H_2O$ (4:1:5) and EtOAc–$C_5H_5N$–$H_2O$ (2:1:2). The aglycone from the organic phase turned brown on exposure to air and showed strong streaking on Si gel and cellulose plates.

*Methylation of erioside and acid hydrolysis.* Erioside (80 mg) was refluxed with DMS (1.0 ml) and $K_2CO_3$ in $(Me)_2CO$ in $N_2$ atmosphere for 4 hr. Usual work-up yielded a brown residue (74 mg) which was refluxed with 2 N HCl–EtOH for 2 hr. After dilution with $H_2O$ and extraction with EtOAc, a residue (28 mg) was obtained, which showed single spot, $R_f$ 0.5 ($CHCl_3$–MeOH–$H_2O$, 35:5:2), and crystallized from MeOH, mp 149°. $^1$H NMR: δ 3.80, 3.81 (2 OMe), 6.0 (1H, *d*, *J* = 10 Hz, C-3), 6.22 (1H, *s*, C-5), 7.84 (1H, *d*, *J* = 10 Hz, C-4).

*Methylation of eriosin dimethyl ether.* Eriosin dimethyl ether (10 mg) in MeOH was reacted with ethereal $CH_2N_2$ at 4°. The derivative crystallized from MeOH, mp 104°.

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