EXPERIMENTAL

Mps are uncorr. NMR spectra were recorded at 100 MHz using TMS as int. standard.

Garuganin III (**2a**). Colourless needles, mp 176–177°. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 215, 250 and 275. IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 1670, 1590, 1570, 1500, 1270, 1245, 1210, 1030, 890, 880 and 810. (Found: C, 71.99; H, 6.57. $C_{22}H_{24}O_5$ requires: C, 71.74; H, 6.51 %.)

Hydrolysis. A MeOH soln of **2a** (12 mg in 5 ml MeOH) containing a drop of conc. HCl was refluxed for 4 hr at 100°. The solvent was removed under red. pres. and HCl was removed under vacuum. The single product (**2b**) obtained was recrystallized from MeOH, mp 149–150°. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 220 and 273 $\lambda_{\max}^{\mathrm{MeOH+AlCl_3}}$ nm: 220 and 300; $\lambda_{\max}^{\mathrm{MeOH+AlCl_3+HCl}}$ nm: 220, 300. IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 3450, 1615, 1590, 1570, 1510, 1380 and 1220.

Demethylation. Compound **2a** (25 mg) was dissolved in dry $CHCl_3$ (15 ml). The mixture was cooled to 0° and BBr_3 (1.5 ml) added dropwise with stirring. The resulting mixture was stirred for 5 hr and allowed to warm to room temp. The solvent was evaporated followed by coevaporation with MeOH (3 × 10 ml) to remove B as methyl borate. The reddish residue obtained was dried under vacuum. Further purification was done by recrystallization from MeOH giving brownish needles (15 mg) of **2c**. Mp 190–191°. UV $\lambda_{\max}^{\mathrm{MeOH}}$ nm: 219, 278 and 285. IR $\nu_{\max}^{\mathrm{Nujol+CHCl_3}}$ cm^{-1}: 3560, 3380, 1610, 1590, 1540, 1460, 1455, 1380, 1220, 1020, 930 and 860.

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STRUCTURE REVISION OF THE COUMARIN, CEYLANTIN

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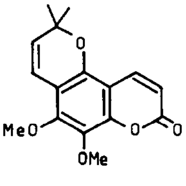
Key Word Index—*Atalantia ceylanica*; Rutaceae; 5,8-dimethoxy-6,7-pyranocoumarin.

Abstract—Ceylantin, isolated from *Atalantia ceylanica*, has been found to be identical with racemosin, isolated from *A. racemosa*, and the revised 5,8-dimethoxy-6,7-pyranocoumarin structure confirmed by nuclear Overhauser experiments.

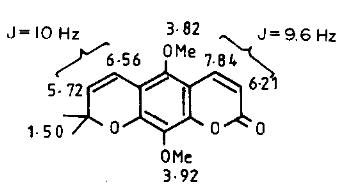
A $C_{16}H_{16}O_5$ coumarin, ceylantin, mp 126–127° was recently isolated from the heartwood of *Atalantia ceylanica* [1]. The ^{1}H NMR spectrum revealed a pyranocoumarin nucleus and two methoxyl groups but irradiation of H-4 was reported to have no effect on the intensity of either methoxyl signal thereby precluding attachment at C-5 for one of them. Ceylantin was accordingly formulated as the angular pyranocoumarin (**1**).

In 1978, racemosin, mp 125–126°, having the same molecular formula as ceylantin, was isolated from the stem and leaves of *A. racemosa* [2]. The linear pyranocoumarin structure **2** deduced from spectroscopic evidence, was supported by a synthesis. The close similarity in physicochemical properties and botanical origin of ceylantin and racemosin led us to suspect that they might possess the same structure. Direct comparison [mmp, TLC, IR, UV ($\lambda_{\max}$ 222, 262 (sh), 270, 325 nm), ^{1}H and ^{13}C NMR] has now confirmed their identity. Moreover, nuclear Overhauser experiments have revealed that each possesses structure **2**. Separate irradiation of the methoxyl signals at δ 3.82 and 3.92 has shown, in each case, that only the former gives an increase in the integrated intensity of both the H-4 and H-4′ signals, by 7% and 7.2%, respectively, consonant with a C-5 methoxyl group on a linear pyranocoumarin.

It has been asserted that trioxygenated coumarins are rare in the Rutaceae and when present the oxygenation is specifically 5,7,8 in *Ruta* and 6,7,8 in *Zanthoxylum* [1].



1



2

The literature reveals however the presence of trioxygenated coumarins in 31 of the 50 genera which have been studied [3]. Moreover, although 5,7,8-trioxygenated coumarins have been isolated from seven of the eight *Ruta* examined, 6,7,8-trimethoxycoumarin has also been reported from three of these [3, 4]. The same coumarin has indeed been found in three species of *Zanthoxylum* [3,5,6] but isopimpinellin (5,7,8) has been isolated from *Z. belizense* and *Z. suberosum* [3], phellopterin (5,7,8) and pimpinellin (5,6,7) from *Z. usambarense* [7] while fraxinol (5,6,7) and 5,6,7-trimethoxycoumarin occur in *Z. integrifolium* [5].

EXPERIMENTAL

IR spectra were recorded in CCl_4, UV spectra in EtOH and NMR spectra in $CDCl_3$.

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4α-METHYLERGOSTA-8,24(28)-DIEN-3β-OL, A MINOR STEROL IN THE SEED OF HORSE CHESTNUT

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Key Word Index—*Aesculus hippocastanum*; Hippocastanaceae; seed oil; unsaponifiable matter; 4α-methyl sterol; 4α-methylergosta-8,24(28)-dien-3β-ol.

Abstract—From ripe horse chestnut seed the 4α-methyl sterol fraction was isolated representing 4.5% of the unsaponifiable matter, i.e. 3 mg% of the seed. This fraction was investigated by capillary GC and combined GC–MS. It contains at least 12 components, of which 5 were identified as: obtusifoliol, 4α-methylergosta-8,24(28)-dien-3β-ol, gramisterol, cycloeucalenol and citrostadienol. The distribution of these five 4α-methyl sterols in the seed was also determined and they represent about 90% of the investigated fraction. 4α-Methylergosta-8,24(28)-dien-3β-ol up to now been found in higher plants only in traces, while in this fraction it was found in the amount of about 5%.

INTRODUCTION

According to present knowledge, the biosynthesis of stigmasterol and sitosterol, the two most common sterols in higher plants, takes place via 4-methyl sterols in such a generally accepted way that obtusifoliol (**2**) is a precursor of gramisterol (**4**) [1]. Only recently Schmit and Benveniste [2] while investigating sterol biosynthesis detected in bramble cell culture traces of 4α-methylergosta-8,24(28)-dien-3β-ol (**3**) and suggested that in higher plants, obtusifoliol (**2**) is first demethylated in position C-14 to give **3** and that the Δ^8 double bond of **3** is then isomerized to yield **4** (Scheme 1). 4α-Methylergosta-8,24(28)-dien-3β-ol (**3**) had been previously found in yeast [3, 4] and in some types of fungi [5], but not in higher plants. However, up to now few experimental data have been published which could confirm this theory and accordingly we investigated the 4-methyl sterol fractions of certain plant oils [6]. We succeeded in detecting traces of **3** only in rapeseed oil, which does not completely confirm the accepted hypothesis of the participation of **3** in sterol biosynthesis. Our investigations were continued with horse chestnut seed which, regarding the quantitative ratio of the Δ^5 and Δ^7 sterols [7] shows considerable deviations from the ratio determined for most higher plants [8]. In this paper we present the results of qualitative and quantitative analysis of the 4-methylsterol fraction of horse chestnut seed.