

## LIMONOIDS FROM *EKEBERGIA PTEROPHYLLA*

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**Key Word Index**—*Ekebergia pterophylla*, Meliaceae, limonoids, ekebergin, methyl angolensate

**Abstract**—The seed of *Ekebergia pterophylla* contains limonoids closely related to methyl angolensate, and similar to those of other *Ekebergia* species. This serves as evidence to support the taxonomic position of the species which has been disputed.

### INTRODUCTION

*Ekebergia pterophylla* (C DC) Hofm is a large shrub or small tree common in Natal on outcrops of the Table Mountain sandstone. The fruit differs from those of other *Ekebergia* species in possessing endosperm, for which reason it has been transferred to the genus *Trichilia* by some authors [1, 2]. In their recent monograph [3] Pennington and Styles include *E. pterophylla* as one of the four species they recognise in the genus *Ekebergia*, pointing out that endospermous seeds occur sporadically throughout the subfamily Melioideae.

Chemically, we have shown that the genera *Trichilia* and *Ekebergia* differ widely. The two species of *Ekebergia* that we have examined [4, Connolly, J D and Taylor, D A H, unpublished work] contain further oxidized derivatives of methyl angolensate, containing a characteristic 15 $\beta$ -hydroxyl group, otherwise unknown in limonoids with a ring D lactone. In contrast, all African species of the large genus *Trichilia* that have so far been examined contain limonoids of the havanensin or prieurianin groups, with a pentacarboxylic ring D [5]. Chemical examination of *E. pterophylla* therefore should provide a good test of its affinities.

### RESULTS AND DISCUSSION

We have now investigated the seed chemistry of *Ekebergia pterophylla*. We find that it contains a complex mixture of limonoids, of which only one was obtained crystalline. This, named E P 1, had the formula  $C_{29}H_{38}O_9$ , mp 241–244°,  $[\alpha]_D^{20} - 76^\circ$ .

The nuclear magnetic resonance spectra showed most of the features associated with methyl angolensate, in addition there were an extra acetoxy group and a secondary hydroxyl, while the ketonic carbonyl was missing. The  $^1H$  NMR spectrum revealed the presence of a chain of three coupled CH groups, consistent with the structure 1. This is extremely similar to the structure of ekebergin (2) previously isolated from *E. capensis*.

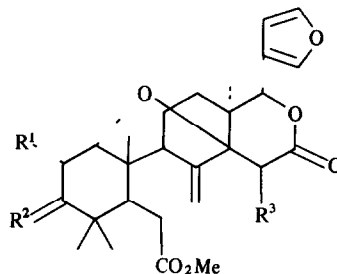
A second compound, E P 2, not obtained crystalline, was identified with the partially synthetic 3 $\alpha$ ,15 $\beta$ -diacetoxy-3-dihydro methyl angolensate (3) [6], by comparison of the  $^1H$  NMR spectra. A third compound, E P 3, also not obtained crystalline, had a  $^1H$  NMR spectrum

very similar to that of ekebergin, and may be the acetate (4) corresponding to ekebergin, although the amount isolated was insufficient to confirm this.

It is hoped to extend these investigations when more seed becomes available, meanwhile the close similarity of E P 1 and E P 2 to ekebergin provides strong supporting evidence for the taxonomic views of Pennington and Styles.

### EXPERIMENTAL

Minced seed of *E. pterophylla* (118 g, collected at Oribi Gorge, herbarium specimen DAHT 316 at Oxford) was extracted in the usual manner. The extract was partitioned between aq. MeOH (3  $\times$  200 ml, 5%  $H_2O$ ) and hexane to remove seed oil, and the limonoids recovered from the concd MeOH layers with  $CH_2Cl_2$ . Evaporation gave the extract (2.8 g). This was chromatographed on silica gel, eluting with EtOAc in  $CH_2Cl_2$ . Further processing of the fractions by prep. TLC gave E P 1 (37 mg) as a crystalline solid, mp 241–243°,  $[\alpha]_D^{20} - 76^\circ$  [Found C, 65.3, H, 7.1%,  $m/z$  530.2522.  $C_{29}H_{38}O_9$  requires C, 65.6, H, 7.2%,  $m/z$  530.2514].  $^1H$  NMR  $\delta$  7.42, 7.37 (H-21, 23), 6.38 (H-22), 5.50 (s, H-17), 5.10 (m,  $W_{1/2} = 8$  Hz, H-2), 5.12, 4.85 (2H-30), 3.71 (CO<sub>2</sub>Me), 3.58



- 1  $R^1 = OAc$ ,  $R^2 = H, \alpha OH$ ,  $R^3 = H$
- 2  $R^1 = CO_2iBu$ ,  $R^2 = H, \alpha OH$ ,  $R^3 = OAc$
- 3  $R^1 = H$ ,  $R^2 = H, \alpha OAc$ ,  $R^3 = OAc$
- 4  $R^1 = OAc$ ,  $R^2 = H, \alpha OH$ ,  $R^3 = OAc$

(2H, *m*, H-1, 3), 2.12 (OAc), 2.05, 2.092 (CMe)  $^{13}\text{C}$  NMR  $\delta$  174.2 s, 170.6 s, 169.6 s, 145.6 s, 142.8 d, 140.8 d, 120.8 s, 112.0 t, 109.8 d, 81.8 s, 80.4 d, 77.9 d, 75.5 d, 68.3 d, 51.9 d, 51.2 q, 45.5 s, 41.3 s, 40.3 s, 35.3 d, 33.5 t, 32.1 t, 29.6 t, 27.7 q, 23.8 t, 21.4 q, 21.2 q, 20.9 q, 14.1 q

Oxidation with Jones' reagent gave the corresponding ketone in the  $^1\text{H}$  NMR spectrum of which H-1 and H-2 appeared as doublets  $\delta$  5.60 (d, H-2, *J* = 4 Hz), 3.70 (d, H-1). Acetylation with pyridine- $\text{Ac}_2\text{O}$  produced no change

Another fraction gave EP2, not obtained quite pure ( $^1\text{H}$  NMR identical with the spectrum of a partially synthetic sample [6]), while a third gave EP3, apparently pure but not crystalline [ $^1\text{H}$  NMR  $\delta$  7.42, 7.36 (H-21, 23), 6.38 (H-22), 5.39 (H-17), 5.29, 5.13 (2H-30), 4.64 (H-15), 3.69 ( $\text{CO}_2\text{Me}$ ), 1.04, 0.97, 0.91, 0.86 (4  $\times$  CMe)]

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## COMPOSITION OF THE STEROL FRACTION IN HORSE CHESTNUT

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**Key Word Index**—*Aesculus hippocastanum*, Hippocastanaceae, sterols

**Abstract**—The  $\Delta^7$ - and  $\Delta^5$ -sterol fractions were isolated from the unsaponifiable matter of ripe, air dried, chestnut seed. The  $\Delta^7$ -sterol fraction amounted to 18.4% and the  $\Delta^5$ -sterol fraction amounted to 8.2% of the unsaponifiable matter. In the  $\Delta^7$ -sterol fraction three components were identified as  $\Delta^7$ -campesterol,  $\alpha$ -spinasterol and  $\Delta^7$ -stigmastenol. One component was probably  $\Delta^5$ -stigmastadienol and five components remained unidentified.  $\Delta^7$ -stigmastenol and  $\alpha$ -spinasterol were the major components and amounted to 74.8% of the fraction. In the  $\Delta^5$ -sterol fraction at least ten components were found. Five of them were identified as cholesterol, campesterol, stigmasterol, sitosterol and  $\Delta^4$ -stigmasten-3-one. Stigmasterol and sitosterol amounted to 73.6% of the fraction.

One of the first important results obtained in the investigation of the constitution of horse chestnut was the isolation of escine present in the mixture of acylated triterpene glycosides of the  $\beta$ -amirin type [1, 2]. Flavonoid glycosides were identified later [3–6]. Fiedler and Hildebrand [7] isolated adenine, adenosine, guanine and uric acid. Damm [8] identified filoguinone in horse chestnut seed and Haenel [9] identified vitamin B com-

plex, methionine and holine. Windaus and Bock [10] first isolated a mixture of sterols in which they identified provitamin D in less than 2% of the mixture, and Allan and coworkers [11] succeeded in isolating  $\alpha$ -spinasterol, butyrospermol, fridelin, taraxasterol and triacontane. The unsaponifiable constituents of horse chestnut oil remained largely unidentified, and although they constitute only a very small part of the oil (2–3%), they comprise of sterols, 4-methylsterols, triterpenes and aliphatic alcohols, vitamins, hydrocarbons, pigments, etc. Recently the composition of the sterol fractions of some vegetable oils have been analysed.  $\Delta^5$ -Sterols, such as stigmasterol, campesterol, sitosterol and  $\Delta^5$ -avenasterol were found to be the major components of most vegetable oils, while in the  $\Delta^7$ -sterol fractions  $\alpha$ -spinasterol and  $\Delta^7$ -stigmastenol mostly predominated [1–6]. In the present study the sterol fraction obtained from a ripe seed dried in air was investigated by GC/MS. The  $\Delta^7$ -sterol fraction amounted to 17% of the unsaponifiable matter which is 0.01% of the dry weight of the seed. On the basis of methylene indices

Sterol nomenclature used in this paper: cholesterol = cholest-5-en-3 $\beta$ -ol, campesterol = (24R)-24-methylcholest-5-en-3 $\beta$ -ol, stigmasterol = (24S)-24-ethylcholest-5,22-dien-3 $\beta$ -ol,  $\Delta^7$ -campesterol = (24R)-24-methylcholest-7-en-3 $\beta$ -ol, sitosterol = (24R)-24-ethylcholest-5-en-3 $\beta$ -ol,  $\alpha$ -spinasterol = (24S)-24-ethylcholest-7,22-dien-3 $\beta$ -ol,  $\Delta^5$ -stigmastadienol = (24R)-24-ethylcholest-5,7-dien-3 $\beta$ -ol,  $\Delta^4$ -stigmasten-3-one = (24R)-ethylcholest-4-en-3-one,  $\Delta^7$ -stigmastenol = (24R)-24-ethylcholest-7-en-3 $\beta$ -ol