Phytochemistry, Vol. 20, No. 9, pp. 2263–2265, 1981.
Printed in Great Britain.

0031–9422/81/092263–03 $02.00/0

EKEBERGIN, A LIMONOID EXTRACTIVE FROM *EKEBERGIA CAPENSIS*

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(*Revised received* 5 *February* 1981)

Key Word Index—*Ekebergia capensis*; Meliaceae; limonoids; ekebergin; methyl angolensate; NMR.

Abstract—Seeds of *Ekebergia capensis* contain a crystalline limonoid to which the name ekebergin has been given. This has been identified as a diacylated methyl angolensate derivative.

INTRODUCTION

Ekebergia is a small genus of African trees, belonging to the Meliaceae, tribe Trichiliae. The type species *E. capensis* is a fairly large tree, widespread in eastern Africa from the Sudan to the Cape. The timber is not durable, but the tree is cultivated for shade and as an ornamental on account of its striking clusters of cherry-like but inedible red fruit. We have examined the bark and timber without finding limonoids; the intensely bitter seeds yielded a group of related limonoids, the constitution of which we now report.

RESULTS AND DISCUSSION

Extraction of the minced seed with refluxing hexane gave an oil (*ca* 20%) with an unpleasant smell, similar to that of *Cedrela odorata*. This was partitioned between aqueous methanol and hexane, the hydrophilic fraction (1%) was then chromatographed over silica gel giving a crystalline solid, ekebergin (0.1%), $C_{34}H_{46}O_{11}$, mp 250°.

Quantitative hydrolysis [1] showed one equivalent of lactone, one equivalent of non-volatile acid, present in the original as a Me ester, and one equivalent each of acetic and 2-methylbutyric acids. One of the remaining O atoms is present in a furan ring, another as a OH group, the third, which corresponds to two C–O signals in the ^{13}C NMR spectrum, must be present as an ether. The ^{13}C NMR spectrum also shows the presence of a vinyl group, which suggests a ring-B cleaved limonoid.

The ^1H NMR spectrum shows the presence of resonances due to a conjugated chain CHOR·CHO(acyl)·CHOH, not further coupled, and two singlets at δ 5.52 and 5.84. Oxidation of ekebergin gives a ketone, in which the CHOH resonance is missing; acetylation gives a monoacetate in which it is shifted downfield. Taken together with a singlet carbon resonance at δ 83.9, this indicates the presence of a Me angolensate type 1–14-ether, with a 3-OH and a 2-acyloxy group. The second acyloxy group, linked to a sharp singlet resonance at δ 5.89 can only be at C-15. In agreement with this, no AB doublet, expected for an unsubstituted 2 H-15, can be found in the ^1H NMR spectrum at 200 MHz. Ring D lactone limonoids acylated at C-15 have not previously been found, but have been prepared by partial synthesis [2, 3].

Table 1. ^1H NMR spectra of ekebergin derivatives (chemical shifts in ppm from internal TMS, couplings (in parentheses) in Hz)

Proton	1*	2†	3‡	4‡
1	3.51 *m*	3.97 (*d* 4.8)	4.20 (*d* 4.9)	3.88 (*d* 6.8)
2	NA	5.19 *m* (4.8) (2.4)	5.75 (*d* 4.9)	5.20
3	4.75 *m*	3.54 (*d* 2.4)(D_2O)	NA	5.20
15	5.84	5.89	5.91	5.91
17	5.66	5.52	5.58	5.82
30A	4.79	4.84	4.89	4.83
30B	5.12	5.22	5.27	5.24
α-Furan A	7.39	7.42	7.43	7.46
α-Furan B	7.39	7.48	7.43	7.46
β-Furan	6.37	6.42	6.37	6.45
CO_2Me	3.65	3.75	3.76	3.76
Ac	2.05	2.21	2.22	2.21
	2.12			2.21
C–Me	0.8	1.00	1.38	1.21
	0.8	1.08	1.25	1.10
	0.96	1.08	1.11	1.03
	0.96	1.11	1.11	0.94
$(Me)_2 \cdot CH$	NA	1.24 (*d* 7)	1.27 (*d* 6.8)	1.20 (*d* 6.5)
$Me \cdot CH_2$	NA	0.96 (*t* 6.7)	—	—

* Partially synthetic [3].

† Determined at 200 MHz.

‡ Determined in $CDCl_3$ at 30° on a CFT20 spectrophotometer at 80 MHz.

NA, not applicable; —, not recorded.

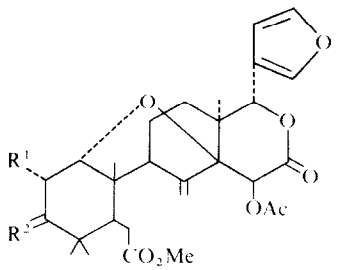
Conformational analysis of the complex methyl angolensate system is difficult, but the spectral data are consistent with 1α,2α,3α-substitution, which we believe to be correct. This is also consistent with the fact that oxidation to a 3-ketone gives a product which does not epimerize.

Assignment of the configuration at C-15 is more difficult. The substituent presumably comes from a 14β–15β-oxide, as does the partially synthetic analogue **1** [3], but the mechanism is obscure. We consider that the most likely structure is that with a 15β-acyloxy group, as a 15α-group would be expected to cause a considerable downfield shift of the resonance of the α-Me group at C-13, and of the vinyl protons, which is not observed. The most probable mechanism for forming this, as for the synthetic analogue **1**, would seem to be opening of the oxide ring by a double inversion at C-14.

Attempted partial hydrolysis to determine the location of the two acyl groups was unsuccessful. However, an impure sample of ekebergin, which contained a homologue with an isobutyrate residue in partial replacement of 3-methylbutyrate showed a slight doubling of the bands due to H-2. This is characteristic of ester replacement in analogous compounds, and we consider therefore that the 3-methylbutyrate ester in ekebergin is at C-2 and the acetate at C-15. We therefore propose that ekebergin has the structure **2**.

The non-crystalline part of the extract gave a ^{1}H NMR spectrum which was similar to that of ekebergin, except for doubling of some bands. It appeared, therefore, that it contained the same nucleus but with different acyl groups. Oxidation of a sample of the non-crystalline fraction gave 50% of a highly crystalline mixture, with a spectrum very similar to that of the oxidation product of ekebergin, **3**. Quantitative hydrolysis of this gave isobutyric, 3-methylbutyric, and acetic acids in the ratio 2:5:12.5; no other acids were detected. It seems probable that it is a mixture of ekebergin, the corresponding isobutyrate and the corresponding diacetate, possibly also including compounds with the higher ester at C-15. It is not known whether the original material contained some of the 3-ketone, or if this was all produced by oxidation.

The mother liquor from the crystalline oxidation product showed a spectrum very similar to that of ekebergin acetate, **4**; therefore, it seems probable that the limonoids of *E. capensis* consist mainly of ekebergin, the corresponding isobutyrate and acetate, and 3 acyl derivatives of these compounds, possibly together with some of the corresponding 3-keto compounds. The ekebergolactones, obtained from the timber of *E. senegalensis* by Ekong and Fasina [4] appear to be more highly oxidized derivatives of ekebergin [5].



1 R^1 = H, R^2 = H, 7OAc
2 R^1 = iBuCO$_2$, R^2 = H, 7OH
3 R^1 = iBuCO$_2$, R^2 = O
4 R^1 = iBuCO$_2$, R^2 = H, 7OAc

In previous investigations of the chemistry of the Trichiliae, only species of *Trichilia* have been examined. African species contain complex limonoids of the prieurianin type, in which ring B is fissioned, while ring D remains carbocyclic, usually oxidized at C-15, either to a carbonyl or to an acyloxy group, possibly in the 15α configuration [6]. The tribe appears to be very close to the Guareeae, many of which contain closely similar compounds. One of these, aphanastatin [7], contains a 2α-acyloxy group. American species of *Trichilia* and African species of *Guarea* contain ring D lactones, but the only Me angolensate derivatives so far obtained from either tribe are from *Guarea thompsonii* [8].

It seems that the limonoids of *Ekebergia* are not far removed from the general pattern found in the Trichiliae, in which highly oxidized ring B fissioned limonoids appear to be the most common terpenoid constituents.

EXPERIMENTAL

Seed of *E. capensis* Sparrm. (5 kg) was minced and extracted with refluxing isohexane. The extract was concd to give a yellow oil which was diluted with isohexane (1.5 l.) and extracted with aq. MeOH (4 × 500 ml, 90%). The MeOH extracts were concd to 500 ml, washed with isohexane, diluted with CH_2Cl_2 (250 ml) and washed with H_2O (3 × 100 ml). The organic layer was then evaporated to give the total limonoid extract (55 g). This was chromatographed over Si gel using EtOAc–hexane mixtures for elution.

Early fractions gave crystalline material, recrystallized (MeOH–CH_2Cl_2) to give ekebergin (**2**) (3.37 g), mp 248–250°, $[\alpha]_D^{23}$ −37° [Found: M^+ 630.3035; $C_{34}H_{46}O_{11}$ requires: 630.3040; ν_{max} 1720, 1760 cm^{-1} (α-acyloxy-lactone [3]). ^{13}C NMR spectrum: 175.8 *s*, 174.0 *s*, 169.9 *s*, 167.3 *s*, 142.9 *s*, 142.9 *d*, 140.8 *d*, 120.5 *s*, 112.3 *t*, 109.7 *d*, 83.9 *s*, 80.5 *d*, 78.5 *d*, 78.3 *d*, 68.6 *d*, 68.1 *d*, 51.6 *q*, 51.4 *d*, 44.9 *s*, 44.3 *s*, 40.5 *d*, 40.0 *s*, 35.4 *d*, 32.1 *t*, 29.9 *t*, 27.6 *q*, 26.5 *t*, 24.0 *t*, 21.9 *q*, 21.2 *q*, 20.1 *q*, 15.8 *q*, 14.5 *q*, 11.25 *q*. Subsequent fractions were impure, but had similar TLC and spectral properties.

Oxidation of ekebergin with Jones reagent gave the corresponding ketone (**3**), mp 250–252°, $[\alpha]_D^{23}$ −25°. Found: M^+ 628.2845; $C_{34}H_{44}O_{11}$ requires: 628.2833. ^{13}C NMR spectrum: 205.2 *s*, 176.0 *s*, 173.3 *s*, 169.6 *s*, 167.0 *s*, 142.9 *s*, 142.9 *d*, 140.8 *d*, 120.5 *s*, 112.15 *t*, 109.8 *d*, 83.8 *s*, 82.9 *d*, 79.9 *d*, 70.1 *d*, 68.75 *d*, 51.8 *q*, 51.5 *d*, 48.5 *s*, 44.5 *s*, 44.3 *s*, 43.2 *d*, 40.7 *d*, 32.8 *t*, 30.0 *t*, 26.5 *t*, 24.4 *q*, 24.4 *t*, 21.8 *q*, 21.7 *q*, 20.3 *q*, 16.0 *q*, 14.7 *q*, 11.3 *q*.

The acetate **4** (prepared with *p*-toluenesulfonic acid catalyst) had mp 282–283°, $[\alpha]_D^{23}$ −53° Found: M^+ 672.3152, $C_{36}H_{48}O_{12}$ requires: 672.3145, ^{13}C NMR spectrum: 175.8 *s*, 174.1 *s*, 170.0 *s*, 169.8 *s*, 167.6 *s*, 143.3 *d*, 143.0 *s*, 140.3 *d*, 121.0 *s*, 112.1 *t*, 109.6 *d*, 83.1 *s*, 80.4 *d*, 76.9 *d*, 76.9 *d*, 68.8 *d*, 66.1 *d*, 51.7 *q*, 51.7 *d*, 45.1 *s*, 44.7 *s*, 40.5 *d*, 39.2 *s*, 36.4 *d*, 31.8 *t*, 30.0 *t*, 27.1 *q*, 26.2 *t*, 24.2 *t*, 22.1 *q*, 21.5 *q*, 21.1 *q*, 20.2 *q*, 15.6 *q*, 14.2 *q*, 11.3 *q*.

Limonoid fractions from chromatography were combined (33.6 g) after discarding some oil. An aliquot (3.7 g) was oxidized with Jones reagent, giving a crystalline solid (1.82 g), mp 250–270°, similar to ekebergin ketone but differing in its ^{13}C NMR spectrum.

Acknowledgements—We are grateful to the Headmaster of Grey's Junior School, Port Elizabeth, for the collection of the seed by his students, and to Dr. F. M. Dean, University of Liverpool, for the 200 MHz spectrum of ekebergin.

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