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ERYTHRINASINATE, AN ESTER FROM THREE *ERYTHRINA* SPECIES*

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Key Word Index—*Erythrina senegalensis*; *E. glauca*; *E. mildbraedii*; Leguminosae; erythrinasinate; pterocarpan; erythrabyssin-1.

Abstract—The novel ester *n*-octacosanyl-3-hydroxy-4-methoxy cinnamate has been isolated from the stem bark of *Erythrina senegalensis*, *E. glauca* and *E. mildbaedii* and its structure determined by spectroscopic data and degradative studies. The known pterocarpan erythrabyssin-1 has also been isolated from *E. glauca*.

INTRODUCTION

The genus *Erythrina* has been extensively studied for its alkaloid constituents but little attention has been paid to the non-alkaloidal products. Recently, Singh *et al.* [1] reported some work on the non-alkaloidal constituents of some *Erythrina* species and Nakanishi and co-workers [2] have carried out the only serious study on the neutral components of this genus. Because of this ready availability of *Erythrina* plants in Cameroon† and Nigeria, and our interest in medicinal plants [3] we have continued [4–6] our study by investigating the constituents of *E. senegalensis*, *E. glauca* and *E. mildbraedii*. In this paper we describe the isolation and structural determination of a novel cinnamate (erythrinasinate, **1**) from the three plants and the isolation of the pterocarpan, erythrabyssin-1 (**6**) from *E. glauca*.

RESULTS AND DISCUSSION

Silica gel chromatography of the hexane extract of *E. senegalensis* and the chloroform extract of *E. glauca* and *E. mildbraedii* afforded the novel compound for which we propose the name erythrinasinate (**1**). It melted at 75–76° and was shown to have the composition $C_{38}H_{60}O_4$ by elemental analysis and mass spectral data ($[M]^+$ *m/z* 586). It gave a positive phenol test and its IR spectrum showed absorption at 3450 (OH), 1710 (C=O), 1660 (C=C), 1625, 1510, 1480 (aromatic), 1280 and 1160 (C–O) and 725 cm^{-1} [$-(CH_2)_x-$]. Erythrinasinate (**1**) is therefore an aromatic long chain ester with unsaturation on the side chain. Its UV spectrum showed λ_{max} 235 (ε10 600) and 325 nm (ε11 400) which is very similar to that reported by Pearl and Beyer [7] for 4-hydroxy-3-methoxy ethyl cinnamate. Compound **1** is therefore a long chain ester of a cinnamic acid. Further evidence for the presence of a substituted cinnamoyl portion in the molecule was furnished by the mass spectrum which gave a base peak at *m/z* 194 and a strong peak at *m/z* 177. The ^1H NMR spectrum further suggested the presence of a cinnamoyl moiety by the presence of doublets at δ6.20 and 7.55 (J = 16 Hz).

Hydrolysis of **1** gave 1-octacosanol (**3**) [8], mp 82–83°, ($[M]^+$ at 410) and 3-hydroxy-4-methoxy cinnamic acid (**4**) [9], mp 225–226°. Erythrinasinate (**1**) is therefore the novel ester *n*-octacosanyl-3-hydroxy-4-methoxy cinnamate. Its acetate (**2**) was also prepared.

It is noteworthy to draw attention to the close similarity between the data reported here for *n*-octacosanyl-3-hydroxy-4-methoxy cinnamate and that published by Singh *et al.* for the coumarin (**5**). For example, **1** has the same UV and IR spectra data as the **5**. In addition, apart from a few discrepancies in the coupling constants, the ^1H NMR spectral data of the two compounds are virtually identical. Finally, the structure of **5** was only suggested and never confirmed. In the light of the above facts, and in the absence of other data, the occurrence of **5** in *E. stricta* [1] should be regarded as doubtful. In fact no other coumarin has yet been reported from the genus *Erythrina*.

Pearl and Beyer [7] have shown that a number of esters of substituted cinnamic acid are active against microorganisms. The novel ester (**1**) will be tested for possible pharmacological activity. From the chloroform extract of *E. glauca*, the biologically active pterocarpan (**6**), previously isolated from *E. abyssinica* [2] was also isolated.

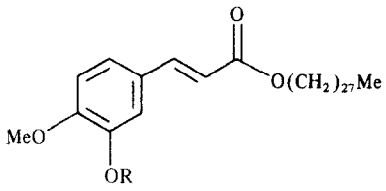
EXPERIMENTAL

Mps (Kofler hot-stage) are uncorr. IR and UV were determined in KBr and MeOH, respectively. MS analyses were performed at 70 eV using a direct inlet system. ^1H NMR spectra were recorded at 90 MHz in $CDCl_3$ with TMS as int. standard.

Plant material. E. senegalensis (D.C.) and *E. glauca* (Wild) were collected at Port Harcourt, Nigeria and Limbe, Cameroon, respectively, while *E. mildbraedii* (Harms) was collected near

* Part 5 in the series "*Erythrina* Studies". For part 4, see ref. [6].

†There are nine identified species of the genus *Erythrina* in Cameroon. Many unidentified species have also been collected by the National Herbarium, Yaounde.



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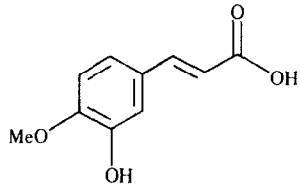
2 R = Ac



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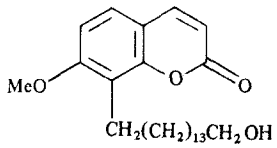

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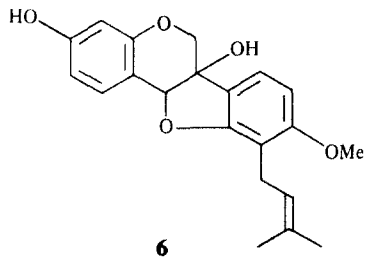

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Yaounde. Herbarium specimens documenting the collection are deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and isolation of compounds. Dried, ground stem bark of *E. senegalensis* (14 kg) was successively extracted with hexane and $CHCl_3$. Some of the hexane extract (75 g) was chromatographed on silica gel (330 g). Elution with hexane gave a fraction which was rechromatographed on silica gel to give crude **1**. It was recrystallized (hexane–CH_2Cl_2) to give **1** (460 mg) as pale white crystals. Dried, ground bark of *E. glauca* (1.5 kg) was extracted with hexane and then $CHCl_3$. The $CHCl_3$ extract (70 g) was chromatographed (silica gel, 460 g) and eluted with hexane–$CHCl_3$ to give a compound which was recrystallized to give **1** (320 mg). Further chromatography gave on elution with hexane–$CHCl_3$ (2:3) an oil which was rechromatographed to give **6** (900 mg). The dried, ground stem bark of *E. mildbraedii* (15 kg) was successively extracted with hexane and $CHCl_3$. The $CHCl_3$ extract (115 g) was chromatographed on silica gel (800 g) to give after recrystallization 2 g of **1**.

Erythrinasinate (**1**). Mp 75–76°. UV λ_{max}^{EtOH} nm (ε): 235 (10 600), 325 (11 400); IR ν_{max}^{KBr} cm^{-1}: 3450, 1710, 1660, 1625, 1510, 1480, 1280, 1160, 725. ^1H NMR: δ0.70 (3H, *t*, Me), 1.20 (52H, *s*, $(CH_2)_{26}$), 3.80 (3H, *s*, MeO), 4.15 (2H, *t*, OCH_2CH_2R), 6.20 (1H, *d*, J = 16 Hz, =CH), 6.85 (1H, *d*, J = 8 Hz, H-6), 6.93 (1H, *s*, exchangeable D_2O, OH), 6.95 (1H, *s*, H-2), 7.05 (1H, *d*, J = 8 Hz, H-5), 7.55 (1H, *d*, J = 16 Hz, CH=). (Found: C, 77.79; H, 11.30. $C_{38}H_{66}O_4$ requires: C, 77.81; H, 11.26.) MS *m/z* (rel. int.): 586 $[M]^+$ (14), 194 (100), 177 (88).

Erythrinasinate acetate (**2**). A sample of **1** was acetylated in Ac_2O–pyridine (1:1, 10 ml) at room temp. for 3 days. Working up in the usual manner followed by recrystallization (CH_2Cl_2) gave **2** (60 mg), mp 80–81°; UV λ_{max}^{EtOH} nm (ε): 234 (9800), 325 (11 000). IR ν_{max}^{KBr} cm^{-1}: 1720, 1700, 1615, 1595, 1285, 1165, 720. ^1H NMR: δ0.70 (3H, *t*, Me), 1.20 (52H, *s*, $(CH_2)_{26}$), 2.30 (3H, *s*, OAc), 3.80 (3H, *s*, MeO), 4.15 (2H, *t*, $COOCH_2$), 6.18 (1H, *d*, J = 15 Hz, =CH), 6.87 (1H, *d*, J = 8 Hz, H-6), 6.98 (1H, *s*, H-2), 7.00 (1H, *d*, J = 8 Hz, H-5), 7.52 (1H, *d*, J = 15 Hz, CH=C). MS *m/z*: 628 $[M]^+$.

Hydrolysis of **1**. A soln of **1** (100 mg) in KOH (10%, 8 ml) and $CHCl_3$ (20 ml) was refluxed for 1 hr. Work up gave **3** (50 mg), mp 82–83° (lit. [8] mp 82.3°), MS *m/z*: 410 $[M]^+$ and **4** 8 mg, mp 225–226° (lit. [9] mp 228°), MS *m/z*: 194 $[M]^+$.

Erythrabissin-1 (**6**). Oil UV λ_{max}^{EtOH} nm (ε): 213 (30 000), 280 (8000), 286 (8000); IR ν_{max}^{KBr} cm^{-1}: 3350, 1610; ^1H NMR: δ1.59 (3H, *s*, Me), 1.69 (3H, *s*, Me), 3.20 (2H, *d*, H-12), 3.70 (3H, *s*, OMe), 3.88 (1H, *d*, H-6), 4.16 (1H, *d*, H-6), 5.16 (1H, *t*, H-13), 5.24 (1H, *s*, H-11a), 6.36 (1H, *d*, H-4), 6.48 (1H, *d*, H-8), 6.50 (1H, *dd*, H-2), 7.14 (1H, *d*, H-7), 7.38 (1H, *d*, H-1), 8.55 (1H, *s*, exchangeable D_2O, OH). MS *m/z*: 354 $[M]^+$. All spectra were identical with those reported in ref. [2].

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(22*R*)-22-HYDROXYCHOLESTERYL ESTERS FROM *NARTHECIUM OSSIFRAGUM*

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Key Word Index—*Narthecium ossifragum*; Liliaceae; (22*R*)-22-hydroxycholesteryl esters; fatty acids.

Abstract—The esters of (22*R*)-22-hydroxycholesterol from the flowers of *N. ossifragum* are monoesters, esterified in the 3β-position only. The main components are cholest-5-en-3β,22*R*-diol 3-caprate (47%) and cholest-5-en-3β,22*R*-diol 3-laurate (44%). The sterol has not been found in the free state in the plant.

INTRODUCTION

22-Hydroxycholesterol has been isolated from the flowering parts of *Narthecium ossifragum* as a major component from the unsaponifiable fraction [1, 2]. This sterol, also named narthesterol [3], was subsequently shown to be the 22*R*-epimer, *viz.* cholest-5-en-3β,22*R*-diol [4, 5].

(22*R*)-22-Hydroxycholesterol has not been found in other plants, but has been identified in human meconium [6] and isolated in very small amounts from bovine adrenal glands [7]. (22*R*)-22-Hydroxycholesterol is an intermediate in the biosynthesis of pregnenolone from cholesterol [8].

In the present study the esters of (22*R*)-22-hydroxycholesterol isolated from *N. ossifragum* have been investigated.

RESULTS AND DISCUSSION

The fatty acid composition of the 22-hydroxycholesteryl esters from *N. ossifragum* is shown in Table 1. The predominant fatty acids are capric acid ($C_{10:0}$) and lauric acid ($C_{12:0}$). The total content of these two acids is 91%, while the amount of the more usual long chain fatty acids (C_{16} and C_{18}) is very low, as is the percentage of unsaturated fatty acids.

The IR spectrum of the purified ester mixture displays the characteristic ester carbonyl absorption at 1735 cm^{-1}. The hydroxyl band at 3430 cm^{-1}, with additional hydroxyl bands at 1021 and 1005 cm^{-1}, show that the esters of 22-hydroxycholesterol from *N. ossifragum* are monoesters with a free hydroxyl group. The absence of a strong hydroxyl band at 1054 cm^{-1}, characteristic for 3β-sterols, indicates that the natural esters are esterified in the 3-position. Mass spectra of the TMSi-derivatives of the esters further support the above assignment, since the spectrum shows a dominant peak at *m/z* 173. This peak is due to the fragmentation of the side chain between C-20

Table 1. Relative amounts of the fatty acids of (22*R*)-22-hydroxycholesteryl esters from *N. ossifragum*

Fatty acid	Weight %
8:0	3
10:0	47
12:0	44
14:0	4
16:0	1
18:0	0.5
18:1	tr*
18:2	tr

*tr = trace (< 0.5%).