Bull. Chem. Soc. Ethiop. **2003**, 17(2), 177-180. ISSN 1011-3924
Printed in Ethiopia

4,5-EPOXIDE-1,6-DIMETHYL-1-VINYLHEXYL *p*-COUMARATE: A NOVEL MONOTERPENE DERIVATIVE FROM *CLEISTOPHOLIS PATENS*

D. Ngnokam[1*], A. Tsopmo[1], J.F. Ayafor[1], J.M. Nuzillard[2] and O. Sterner[3]

[1]Department of Chemistry, University of Dschang, Box 67, Dschang, Cameroon
[2]CNRS-UPRESA 6013, Laboratoire de Pharmacognosie CPCBAT-Bâtiment 18, Moulin de la Housse, BP. 1039, 51097 Reims cedex 2, France
[3]Department of Organic Chemistry 2, Lund Institut of Technology, S 221 00 Lund, Sweden

(Received November 22, 2002; revised July 10, 2003)

ABSTRACT. A novel monoterpene derivative (**1**) and four known partially and total acetylated tri- and tetrarhamonoside dodecanyl ether derivatives: cleistrioside-2 (**2**) and cleistrioside-3 (**3**), cleistetroside-6 (**4**) and cleistetroside peracetate (**5**) have been isolated from the fruits of *Cleistopholis patens.*

KEY WORDS: *Cleistopholis patens,* Annonaceae, Oligosaccharide, Partially acetylated tri- and tetrarhamnoside dodecanyl ether derivatives, Cleistrioside, Cleistetroside, Monoterpene derivative

INTRODUCTION

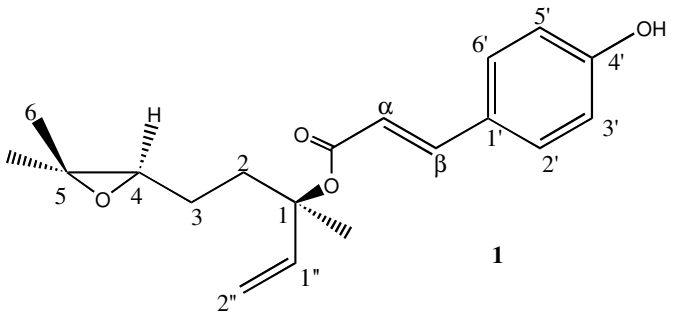
Cleistopholis patens (Benth.) Engl. et Diels (Annonaceae) is a large tree which occurs in the rain forests of Africa, from Sierra Leone to Congo and Uganda [1-3]. The stem bark finds some local use in the treatment of infective hepatitis and stomach disorders, the roots are used as a vermifurge [4] and the leaves are said to remedy fever [5].

Previous phytochemical studies carried out on the stem and root-bark have yielded a number of monoterpenes, sesquiterpenes and alkaloids [6-11]. As part of our study of the family Annonaceae, we report here the isolation and the structural elucidation of a new monoterpene derivative together with four known partially and total acetylated tri- and tetrarhamnoside dodecanyl ether derivatives [12-14].

RESULTS AND DISCUSSION

The novel monoterpene derivative (**1**) and the four known oligo saccharides (**2-5**) were obtained from the methylene chloride extract and purification was achieved by vacuum liquid chromatography and subsequent flash and silica gel column chromatography. Investigation of the non-polar fractions led to the isolation of cleistetroside peracetate (**5**) [12]. Cleistrioside-2 and -3 (**2, 3**) and cleistetroside-6 (**4**) [13, 14] were obtained as gummy solids from the polar fractions. Their structures were determined by means of spectroscopic data and by comparison of physical and spectral data with those reported for the same compounds in the literature [12-14].

*Corresponding author. Tel: (237) 9558830. E-mail: dngnokam@yahoo.fr



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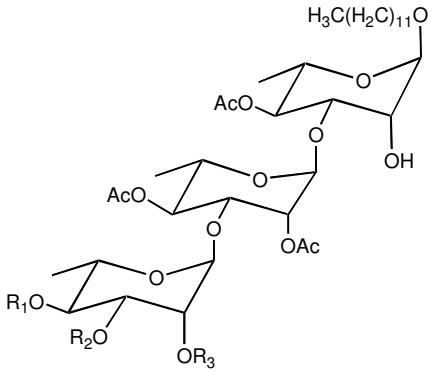
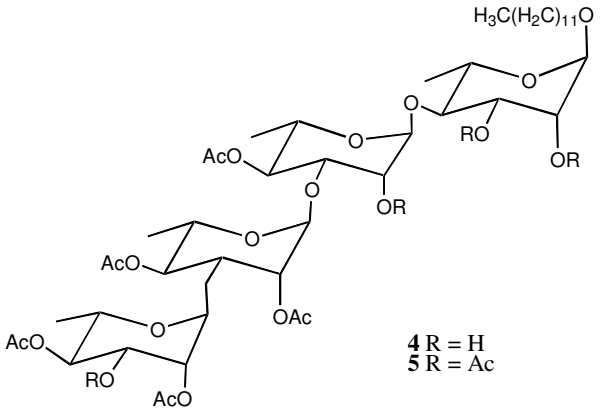


2 $R_1 = R_2 = Ac$, $R_3 = H$
3 $R_1 = R_3 = Ac$, $R_2 = H$



4 R = H
5 R = Ac

Fractions obtained with a mixture hexane-acetone (85:15) were purified over silica gel to yield compound **1** as coloured oil. $[\alpha]_D^{22}$ -4.5° ($CHCl_3$, *c* 3.5). Its UV spectrum showed a significant absorption above 210 nm characteristic of benzene and cinnamoyl chromophores. Its IR spectrum showed absorption bands typical of phenolic hydroxyl group (3342 cm^{-1}), α,β-

unsaturated ester carbonyl (1693, 1514 cm^{-1}), isolated C=C bond (1631 cm^{-1}) and benzene ring (1600 cm^{-1}). The mass spectrum agreed with the $C_{19}H_{24}O_4$ formula. Fragments ions at *m/z* 164 (41%) and 147 (100%) suggests the presence of hydroxycinnamate unit. The ^{13}C NMR displayed signals for 19 carbon atoms: 3 angular methyl groups, 3 methylenes including 2 sp^3 and 1 sp^2, eight methines including seven olefinic and one sp^3 bearing oxygen, 5 quaternary carbons including one ester carbonyl group, two sp^3 bearing oxygen and two sp^2 carbon atoms linked to carbon and oxygen atoms, respectively. This was confirmed by the ^1H NMR spectrum in which three methyls were observed as singlet. Signals attributable to exo-methylene group occurred as a doublet at δ 5.14 and 5.20. The signal observed at δ 2.78 (triplet) was attributed to H-4. The value of the chemical shift indicated that, this methine was included into 1,2-epoxide unit. The AB and AA'BB' systems due to α and β, and aromatic protons appear together between δ 6.18 and 7.52.

In the HMBC spectrum, pertinent correlations observed between the vinylic protons H-2'' and C-1'' and C-1, between H-1'' and C-1, C-2 and C-2'' as well as between H-2 and C-1, C-3 and C-1'' showed that the *p*-coumaroyl moiety was attached to C-1. A series of ^1H-^1H Cosy, in combination with HMBC, HMQC experiments, allowed the assignment of proton resonance for the non-equivalent methylene protons, showing a coupling sequence through two distinctive methylene groups, identified, respectively, as the methylenes in 2 (δ 1.97 and 2.10) and 3 position (δ 1.56 and 1.67).

The relative stereochemistry was determined on the basis of correlations observed from the NOESY spectrum. Important correlations were observed from H-2α to C_1-Me, H-3 and H-4, from H-4 to H-2α and C_5-Me. The stereochemistry of **1** is then as represented in the structure **1**.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectral data for compound **1** in $CHCl_3$.

No	^{13}C	^1H
2''	113.5	5.14 d (J = 11.0 Hz)
		5.20 d (J = 17.5 Hz)
1''	141.1	5.97 dd (J = 11.0 and 17.5 Hz)
1	82.4	-
2	36.2	1.97/2.10 m
3	23.2	1.56/1.67 m
4	64.4	2.78 t (J = 6.4 Hz)
5	59.2	-
6	18.4	1.26 s
$\underline{CH_3}$-C_{-5}	24.6	1.29 s
$\underline{CH_3}$-C_{-1}	23.7	1.59 s
-O-C=O	166.6	-
$C_{-\alpha}$	115.4	6.18 d (J = 15.9 Hz)
$C_{-\beta}$	144.6	7.52 d (J = 15.9 Hz)
1'	125.6	-
2', 6'	129.8	7.33 d (J = 8.6 Hz)
3', 5'	115.9	6.83 d (J = 8.6 Hz)
4'	159.1	-

EXPERIMENTAL

General. Mps: uncorr. IR: NaCl. NMR spectra were recorded at 125 MHz for ^{13}C and 500 MHz for ^1H. Chemical shifts are given in δ value with TMS as internal standard. The ^1H and ^{13}C chemical shifts are summarised in Table 1. EI-MS was measured at 70 eV. TLC was carried out

on silica gel. Compounds were detected by spraying with 50% solution of H_2SO_4 in H_2O following by heating. UV spectra were determined as methanol solution.

Plant material. The fruits of *Cleistopholis patens* were collected in the Korup National Park, Cameroon. A voucher specimen has been deposited at the National Herbarium, Yaounde.

Extraction and isolation. The air-dried and finely powdered fruits of *Cleistopholis patens* (6 kg) were extracted with methylene chloride (19 L) at room temperature. The extract obtained (114 g) was subjected to flash chromatography over silica gel, eluting with hexane, and mixture of hexane-acetone of increasing polarity. Fractions 8-14 (20 g) were pooled and rechromatographed repeatedly on a silica gel column using a mixture of hexane-acetone (85:15) to yield compound **1** (600 mg) and cleistetroside peracetate (**5**) (250 mg). Fractions 17-21 (15 g) were pooled and rechromatographed on a silica gel column, eluting with $CHCl_3$, mixture of $CHCl_3$-MeOH of increasing polarity to afford cleistrioside-2 and -3 (**2, 3**) (30 mg and 150 mg, respectively) and cleistetroside-6 (**4**) (230 mg).

*Compound **1***. Coloured oil. $[\alpha]_D^{22}$ = -4.5° (*c* 3.5, $CHCl_3$). UV λ_{max} (MeOH) log (ε) nm: 211 (4.0), 227 (4.0), 301 (4.2), 312 (4.3). IR ν_{max} ($CHCl_3$) cm^{-1}: 3342 (OH), 1693, 1631, 1600, 1514, 1448, 1375, 1169, 1105, 985. EIMS. (70 eV) *m/z* $[M]^+$ 316 (5), 301 (20), 164 (40), 147 (100), 119 (10), 91 (10), 81 (10).

ACKNOWLEDGMENT

We gratefully acknowledge the AUPELF-UREF (Agence Universitaire de la Francophonie) for the financial support.

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