UVARISESQUITERPENE D: A NEW BENZOPYRANYL SESQUITERPENE ISOLATED FROM *UVARIA LUCIDA ssp. LUCIDA*

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ABSTRACT:
Hitherto unreported benzopyranyl sesquiterpene, uvarisesquiterpene D, has been isolated from the stem bark of Uvaria lucida ssp lucida. Its structure had been elucidated by spectroscopic analysis (IR, MS and NMR) and comparison of its spectral data with the known related compounds uvarisesquiterpene B and selin-11(12)-4α-ol. The latter compound was co-isolated with the new natural product as well as caryophyline oxide and lucidine.

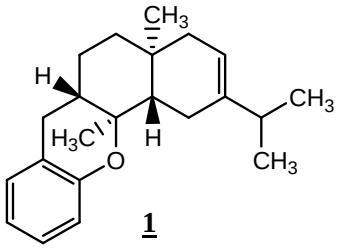
Key words: Annonaceae, uvaria, bezopyranylsesquiterpenes, uvarisesquiterpenes

INTRODUCTION

Uvaria species of the family Annonaceae which are widely distributed throughout tropical Africa had been studied extensively yielding novel compounds of varied structural moieties (Nkunya *et al* 1985, 1990, Nkunya 2005, Qiong Ming XU *et al* 2005, Labouef *et al.* 1982, Elias *et al.* 1988, Hufford *et al.* 1978, 1982, Cole *et al.* 1976, Tammani *et al.* 1970, Anchenbach *et al.* 1997, Weenen *et al.* 1991, 1990, Makangara *et al.* 2002, Wachira 2001) and having a wide range of biological activities (Kihampa 2002, Nkunya *et al.* 1991, Okokon *et al.* 2006, Oluremi *et al.* 2010, Ankisetty *et al.* 2006). In continuation with search for compounds with interesting structural and biological properties from the genus *Uvaria*, studies were carried out on the root back extract of *Uvaria lucida ssp. lucida* and herein is reported the isolation of a new benzopyranyl sesquiterpenoid, uvarisesquiterpene D (**2**). Uvarisesquiterpene D was isolated along with other known compounds, selin-11(12)-en-4α-ol (**3**), caryophyllene oxide (**4**) and guaienol (**6**). Uvarisequiterpene D was isolated in very small amount and thus it was not possible to carry out bioassay experiments on it.

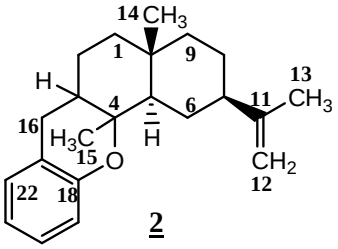
RESULTS AND DISCUSSION

Column chromatography of the petroleum ether extract of the root bark of *Uvaria lucida ssp. lucida* using a gradient of hexane and ethyl acetate gave fractions which contained lucidene, (**5**) (Weenen *et al.* 1990), guaienol (**6**), selin-11(12)-4α-ol (**3**) and uvarisesquiterpene D (**2**), respectively. The fraction containing crude uvarisesquiterpene D was chromatographed repeatedly until one major spot was revealed on TLC. Uvarisesquiterpene D crystallized out from petroleum ether and was recrystallized from petroleum ether affording white needles, mp 130-131 ^{o}C. The Infra-Red spectrum did not exhibit any hydroxyl (OH) absorption but had bands at 1639 cm^{-1} and at 903 cm^{-1} for the isopropene double bond (Frasenus *et al.* 1987). A simple aromatic absorption signal was evident from the IR absorption band at 1601 cm^{-1}.



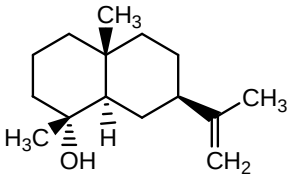
1

Uvarisesquiterpene B



2

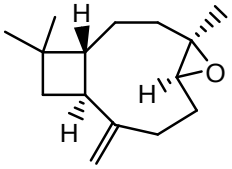
Uvarisequiterpene D



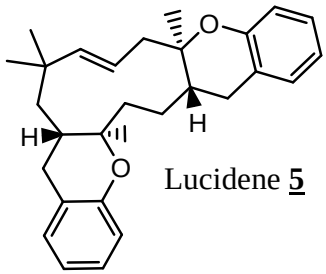
3

Seli-11(12)-en-4α-ol

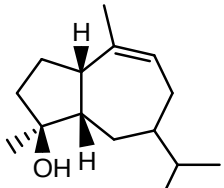
The MS spectrum of the new compound compares well with related known compounds also isolated from *Uvaria species* namely uvarisesquiterpenes A-C (Elias *et al.* 1988) and tanzanene (Weenen *et al.* 1991) with a molecular ion peak at m/z 310 and a base peak at m/z 107 $[C_7H_7O]^+$. Other peaks which appear at m/z 203 $[M-C_7H_7O]^+$, 91 $[C_7H_7]^+$ and 77 $[C_6H_5]^+$ further point towards a benzopyranyl scaffold similar to uvariseqsquiterpenes A-C. The ^{13}C NMR spectrum also indicated close resemblance to uvarisesquiterpene B (Table 1). Whereas the position of the signals for the aromatic carbons (C-17 to C-22) are identical in both uvarisesquiterpenes B and D, the olefinic carbons (C-11 and C-12 in Table 1) for the exocyclic carbon-carbon double bonds in uvarisesquiterpene D matches very well with those of selin-11(12)-en-4α-ol indicating that the novel compound share those structural features with the two compounds.



Caryophyline oxide **4**



Lucidene **5**



Guaienol **6**

Table 1: ^{13}C NMR Chemical Shifts (δ ppm) for uvarisesquiterpene B, uvarisesquiterpene D and selin-11(12)-en-4α-ol

C-	Uvarisesquiterpene B (1)	Uvarisesquiterpene D (2)	Selin-11(12)-en-4α-ol (4)
1	42.69	42.3	43.4
2	23.15	25.9	26.1
3	49.41	49.1	46.4
5	72.95	72.6	72.2
5	51.17	55.4	55.0
6	30.38	30.1	26.9
7	141.75	45.8	44.7
8	116.11	27.0	22.7

C-	Uvarisesquiterpene B (1)	Uvarisesquiterpene D (2)	Selin-11(12)-en-4α-ol (4)
9	42.99	41.3	41.2
10	36.40	37.6	34.6
11	36.86	**150.5**	**150.7**
12	21.16	**108.2**	**108.1**
13	21.67	21.0	21.0
14	13.58	14.4	18.7
15	22.78	22.7	20.2
16	24.89	24.9	
17	**127.84**	**127.9**	
18	**153.83**	**153.9**	
19	**115.14**	**115.2**	
20	**126.90**	**127.0**	
21	**120.16**	**120.2**	
22	**131.36**	**131.4**	

Furthermore, analysis of the ^{1}H NMR gave strong support of similarities of the new compound to uvarisesquiterpene B as well as to that of selin-11(12)-en-4α-ol (Table 2). Three methyl resonances are observed at δ 0.89 ppm attributed to C-14 protons, at δ 1.13 ppm for the C-15 protons (CH_3-C-O) and at δ 1.74 ppm for the C-13 protons in structure **2**. The chemical shift value of the isopropenyl methyl group is close to that of selin-11(12)-en-4α-ol reported by Zalkov [1976] and Huffman [1973]. The two olefinic protons of the isopropenyl group appear as a doublet of doublets at 4.70 ppm (J_1 = 4.7, J_2 = 1.4 Hz). The rest of the ^{1}H NMR spectral data compares well with those of uvarisesquiterpene B. Thus, on the basis of these spectral data the novel compound was assigned structure **2** and given the trivial name uvarisesquiterpene D.

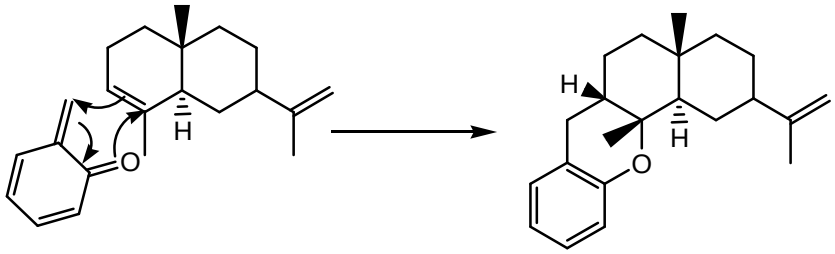
Table 2: Assignment of ^{1}H NMR (300 MHz) spectrum of uvarisesquiterpene D (2).

H	δ (ppm)	multiplicity	J_1 (Hz)	J_2 (Hz)	INTERGRATION
1	1.25 – 1.46	m	-	-	(2H)
2	1.25 – 1.46	m	-	-	(2H)
3	1.90 – 2.10	m	-	-	(1H)
5	1.90 – 2.10	m	-	-	(1H)
6	1.60 – 1.72	m	-	-	(2H)
7	1.90 – 2.10	m	-	-	(1H)
8	1.60 – 1.72	m	-	-	(2H)
9	1.25 – 1.46	m	-	-	(2H)
12	4.70	dd	4.7	1.4	(2H)
13	1.74	s			(3H)
14	0.89	s			(3H)
15	1.13	s			(3H)
16α	1.89	dd	13.0	1.0	(1H)
16β	2.90	dd	13.0	1.0	(1H)
19	6.72	dd	7.2	1.0	(1H)
20	7.02	td	7.4	1.5	(1H)
21	6.82	td	7.6	1.5	(1H)
22	7.05	dd	7.6	1.5	(1H)

The absolute stereochemistry of uvarisesquiterpene D could not be established based on the available spectral data. Therefore, the assigned structure only shows the relative stereochemistry of the compound.

PROPOSED BIOSYNTHETIC ORIGIN OF UVARISESQUITERPENE D

The co-isolation of selin-11(12)-en-4α-ol and presence of β-selinene, detected by GC-MS as 18% of the apolar fraction, is of interest as it may point to the biosynthetic origin of uvarisequiterpene D through a Diels-Alder cyclization reaction of β-selinene with quinone methide hypothesized earlier for lucidene (Weenen *et al.* 1990) and tanzanene (Weenen *et al.* 1991). The hypothesized biosynthesis was later verified by the biomimetic synthesis of lucidene (Adlington *et a*l 1999) as well as tanzanene (Shashikumar *et al.* 1996). For uvarisesquiterpene D, however, this hypothesis could only be asserted with certainty on verifying the stereochemistry at C-3 in uvarisesquiterpene D.



Scheme 1: Possible biosynthetic origin of uvarisequiterpene D

EXPERIMENTAL SECTION

Plant material was collected from Bushiri forest, Pangani district, and identified by the herbarium experts in the Department of Botany, University of Dar es Salaam, where a voucher specimen is deposited (Mwasumbi/Nkunya Coll. No. 14230). The root back was chopped and dried in the shade at room temperature (25-30 °C). The pulverized root bark (800 g) was soaked for three days in petroleum ether (40-60 bp range). Evaporation of ether afforded 44 g of a dark brown gum.

The crude petroleum ether extract (22 g) was fractionated on a vacuum liquid chromatography (VLC) using silica gel and a gradient of petroleum ether and ethyl acetate (100% PE to 10% Ethyl acetate) to afford 17 smaller fractions. The first four fractions appeared similar on TLC and were pooled to give 8.0 g of inseparable oil, GC-MS analysis of which revealed presence of known sesquiterpenes: cyperene (39.3%), β-selinene (18%), α-selinene (5.5%), humulene (1%) and several others in smaller amounts.

Lucidene: TLC analysis of fractions five to nine gave a single spot, thus the fractions were pooled to give 1.2 g from which a solid crystallized out from a 19:1 mixture of PE:EA. GC-MS analysis of the solid revealed it to be a mixture of two compounds. Repeated recrystallization from 10% ethylacetate in petroleum ether finally gave a pure sample which was identified as lucidene (**5**) (Weenen *et al* 1990) mp 209-210 ºC. IR ν cm^{-1} 2997, 1607 (C=C aromatic), 1583, 1485, 1452, 1254 (C-O), 1231, 1206, 1105, 1076, 982 and 930; ^{1}H NMR (300 MHz) δ7.05 (t, *J* = 7.5 Hz, 2H), δ7.04 (d, *J* = 7.5 Hz, 1H), δ6.98 (d, *J* = 7.5Hz, 1H), δ6.80 (t, *J* = 7.5 Hz, 1H), δ6.75

(t, *J* = 7.5 Hz, 1H), δ6.74 (d, *J* = 7.5 Hz, 2H), δ5.63 (ddd, *J* = 16.0Hz, *J* = 7.5Hz, *J* = 7.5Hz, 1H), δ5.76 (d, *J* = 16, 1H), δ2.79 (dd, *J* = 16.0, J = 5.5 Hz, 1H), δ2.67 (dd, *J* = 16 Hz, *J* = 4.5 Hz, 1H), δ2.57 (d, *J* = 7.5Hz, 2H), δ2.56 (dd, *J* = 16.0 Hz, *J* = 11 Hz, 1H), δ2.22 (m, 1H), δ2.08 (vbr s, 1H), δ 1.96 (dddd, *J* = 11.5 Hz, *J* = 5.5 Hz, *J* = 5.5 Hz, *J* = 1.5 Hz, 1H), δ1.89 (br m, 2H), δ1.69 (bm, 1H), δ1.56 (dd, *J* = 14.0 Hz, *J* = 1.5 Hz, 1H), δ1.25 (s, 6H), δ1.12 (s, 3H), δ1.08 (dd, *J* = 14.0, *J* = 5.5 Hz, 1H) and δ1.07 (s, 3H); ^{13}C NMR (75 MHz) δ153.6, 144.0 (2C), 129.2, 128.7, 127.3, 127.1, 124.5, 122.9, 121.8, 119.7, 119.4, 117.3, 116.3, 79.8, 79.5, 49.0, 46.1, 40.8, 35.8, 33.0, 32.7, 32.0, 30.1, 29.2, 26.5, 23.5, 21.2 and 19.8. MS, m/z (% rel. int.) 416 [M+], 309 $[(M-C_7H_7O)]^+$, 171, 159, 107($[C_7H_7O]^+$), 91, 78 and 41.

Selin-11(12)-en-4α-ol: Fraction 10 of the gradient elution from VLC indicated to be a mixture of three compounds. Flash chromatographed with a 9:1 petroleum ether/ethyl acetate eluent afforded a white solid which was recrystallized from petroleum ether to give 140 mg pure compound identified as selin-11(12)-en-4α-ol. The compound has a melting point of 93-95 ºC (literature mp 94-96 ºC, Panichpol *et al* 1978). IR ν cm^{-1} 3578 (O-H), 2994, 2859, 1638 (C=C), 1450, 1384, and 720; ^{1}H NMR (300 MHz) δ4.68 (dd, *J* = 6.2 Hz, *J* = 1.0 Hz, 2H), δ2.15 (s, 1H), δ1.72(s, 3H), δ1.09 (s, 3H) and 0.86 (s, 3H) and ^{13}C NMR (75 MHz) δ150.7, 108.1, 72.2, 55.0, 46.4, 44.7, 43.4, 41.2, 34.6, 26.9, 26.1, 22.7, 21.0, 20.2 and 18.7; and MS, m/z (% rel. int.) 222 ($[M^+]$, 100), 205 (39), 135 (8), 109 (5), 95 (10), 81 (10), 52 (10) and 41 (8).

Uvarisesquiterpene D: Fractions 13-15 contained mixtures which were analyzed by GC-MS only and revealed presence of more uvarisesquiterpene type fragmentations. Fraction 16 of the gradient elution afforded 1.2 g of a mixture which was further chromatographed with 20% EA in PE to give three fractions with fraction 3 giving 17 mg of uvarisesquiterpene D. Yield 0.00174%, mp 130-131°C. IR ν cm^{-1} 2994, 1639, 1601, 1499, 1450, 1384, 1234, 1211, 1087 and 903; ^{1}H NMR (300 MHz) δ 1.25-1.46 m, 6H (H-1, H-2 and H-9), δ 1.90-2.10, m 3H (H-3, H-5 and H-7), δ 1.60-1.72 m, 3H (H-6 and H-8), δ 4.70 dd, J = 4.7, 1.4, 2H (H-12), δ 1.74 s, 3H (H-13), δ 0.89 s, 3H (H-14), δ 1.13 s, 3H (H-14), δ 1.89 dd, J = 13.0, 1.0Hz, 1H (H-16α) δ 2.90 dd, J = 13.0, 1.0 Hz, 1H (H-16β), δ 6.72 dd J = 7.2, 1.0 Hz, 1H (H-19), δ 7.02 td J = 7.4, 1.5 Hz, 1H (H-20), δ 6.82 td J = 7.6, 1.5 Hz, 1H (H-21) and δ 7.05 dd J = 7.6, 1.5 Hz, 1H (H-22); ^{13}C NMR (75 MHz) δ 42.3, 25.9, 49.1, 72.6, 55.4, 30.1, 45.8, 27.0, 41.3, 37.6, 150.5, 108.2, 21.0, 14.4, 22.7, 24.9, 127.9, 153.9, 115.2, 127.0, 120.2, 131.4 and MS, m/z (% rel. int.) 310 ($[M]^+$, 21), 203 ($[M-107]^+$, 29), 133 (12), 121 (10), 107 ($[C_7H_7O]^+$, 100), 91 ($[107-O]^+$, 22), 77 $[C_6H_5]^+$, 45), 67 (18), 55 (24), and 43 (83).

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