

of natural thyransferyl 23-acetate (3)<sup>32</sup> ( $[\alpha]_{220}^{23} +250^\circ$ ,  $[\alpha]_{300}^{23} +27^\circ$  ( $c$  0.02, MeOH)).

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**Supplementary Material Available:** <sup>1</sup>H NMR spectra of 1, 3, 5, 10, 12-15, 19-31, 34, 36-43, 45, 47, 49-54, 56-58, 62-73, 75-80, 83-92, 94, and 96-99 and <sup>13</sup>C NMR spectrum of 45 (78 pages). Ordering information is given on any current masthead page.

## Lucidene, a Bis(benzopyranyl) Sesquiterpene from *Uvaria lucida* ssp. *lucida*

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Lucidene (1), a bis(benzopyranyl) sesquiterpene was isolated from the rootbark of *Uvaria lucida* ssp. *lucida*, and its structure was determined by spectroscopic methods and single-crystal X-ray crystallography. It is optically inactive and crystallizes in enantiomeric pairs. High-resolution <sup>1</sup>H NMR (400 and 600 MHz) spectroscopy allowed the assignment of all protons and indicated the existence of relatively slowly interconverting conformations. The three known dihydrochalcones, uvaretin, diuvaretin, and chamuvaretin, as well as benzyl benzoate were isolated from the same plant as well.

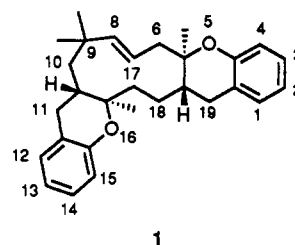
### Introduction

*Uvaria* species have been a source of several new compounds,<sup>1,2</sup> some with cytotoxic,<sup>3</sup> antimicrobial,<sup>4</sup> and antimalarial<sup>5</sup> activity. A typical structural feature that occurs frequently in compounds isolated from *Uvaria* species is the presence of benzyl or *o*-hydroxybenzyl groups, as in the uvaretins<sup>3,6</sup> and some indoles.<sup>7,8</sup> Sesquiterpenes containing *o*-hydroxybenzyl groups occur in *Uvaria* species as well, and three such compounds have thus far been reported.<sup>9</sup> In this paper we describe the determination of the structure of a new sesquiterpene, viz. a bis(benzopyranyl) sesquiterpene, which we have named lucidene (1). The present investigation was carried out as part of our project on antimalarial constituents of *Uvaria* species. Lucidene was isolated from the petroleum ether extract of the rootbark of *Uvaria lucida* ssp. *lucida*, which showed relatively high in vitro activity against the multidrug resistant K<sub>1</sub> strain of *Plasmodium falciparum*; however, lucidene itself showed no such activity.<sup>10</sup>

### Results and Discussion

The title compound (1) was isolated from the petroleum ether extract of the rootbark of *Uvaria lucida* using gradient silica gel chromatography (hexane/ethyl acetate), in addition to the known compounds benzyl benzoate,<sup>8</sup> chamuvaretin,<sup>8,11</sup> uvaretin,<sup>3,8</sup> and diuvaretin.<sup>3,8</sup>

Lucidene (1), which was recrystallized from hexane, showed a mass spectrum with characteristic ions at  $m/e$  416 (M<sup>+</sup>), 309 (monooxybenzyl sesquiterpene: M<sup>+</sup> - hy-



droxybenzyl) and 107 (*o*-hydroxybenzyl). The high-resolution MS showed the M<sup>+</sup> at  $m/z$  416.2720, which corresponds with C<sub>29</sub>H<sub>36</sub>O<sub>2</sub> (calcd 416.2715). These mass spectral data suggest for 1 a sesquiterpene structure containing two oxybenzyl fragments.

The UV ( $\lambda_{max}$  (hexane) 284, 277, 228, 220 nm) and IR spectra (KBr, 1610, 1584 (C=C), 1262, 1246, 1235, 1221 (C—O), 760, and 753 cm<sup>-1</sup> (ortho-substituted phenyl)) suggest the presence of two benzopyran moieties in 1.

Since it was not possible to fully characterize compound 1 with the available spectral data (400- and 600-MHz <sup>1</sup>H NMR, UV, IR, and MS), single-crystal X-ray diffraction

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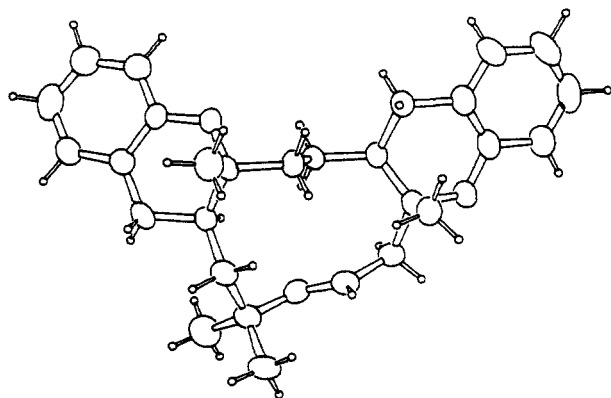


Figure 1.

analysis was carried out. This analysis reveals the presence of two enantiomers, one of which has the structure depicted in Figure 1.

The  $^1\text{H}$  NMR spectrum of 1 taken at 298 K indicates the presence of two ortho-substituted phenyl rings, four methyl groups which appear as singlets, and several methylene and methine protons. Some very broad peaks were observed in the aliphatic region, which were initially contributed to impurities. However, the molecular structure determined by the X-ray analysis pointed to a different interpretation, viz. that these very broad signals may be attributed to slowly interconverting conformations of the undecene ring. This was confirmed by taking the  $^1\text{H}$  NMR spectrum at 328 K, whereby significant sharpening of all broad peaks occurred. At that temperature, the multiplicity of all peaks could be established, except for the signal at  $\delta$  2.05 ( $\delta$  2.08 at 298 K), which was still rather broad. The assignment of most signals, as well as their multiplicity and  $J$  values, were mainly determined by single-frequency decoupling experiments (see Table I). For example, the methine proton at C-10A ( $\delta$  1.96) appears as a ddd ( $J = 11, 5.5, 5.5$  Hz) but actually is a dddd, because decoupling of the signal at 1.55 (H-10 $\alpha$ ) clearly caused line sharpening ( $J \sim 1$  Hz). The methine proton at C-19A ( $\delta$  2.22, m) is structurally very similar to the proton at C-10A, but it is shifted downfield by approximately 0.2 ppm and shows somewhat different coupling constants. Protons at C-6, C-7, and C-8 show a relatively simple ABX pattern, with both protons at C-6 appearing as a single doublet ( $J = 7.5$  Hz) instead of the expected AB system. The C-10 $\beta$  proton resonates at relatively high field ( $\delta$  1.08, dd,  $J = 16, 7.5, 7.5$  Hz), almost hidden under a methyl peak with the same chemical shift. Decoupling of the C-10 $\alpha$  proton, however, clearly revealed its multiplicity, and its coupling with H-10 $\beta$  ( $J = 16$  Hz). Inspection of molecular models suggests that the relatively high field position of H-10 $\beta$  is possibly due to an anisotropic effect of the C-7-C-8 double bond.

The most striking phenomenon in the  $^1\text{H}$  NMR spectra, however, is the remarkable peak sharpening at 328 K, in comparison with 298 K. The two benzopyran moieties apparently decrease the flexibility of the undecene ring system, thereby slowing down conformational interconversion. Some of the signals that are broad in the 600-MHz  $^1\text{H}$  NMR spectrum appear slightly sharper in the 400-MHz spectrum at the same temperature, since the coalescence temperature is dependent on  $|\nu_\alpha - \nu_\beta|$ . The C-19 methylene proton at  $\delta$  2.08 in the 298 K spectrum, is the only proton that still gives a broad signal ( $\delta$  2.05) at 328 K. This implies that the chemical shift of this proton differs significantly more when going from one conformation to another ( $|\nu_\alpha - \nu_\beta|$ ) than the chemical shifts of all other protons.

Table I.  $^1\text{H}$  NMR (600 MHz) Spectral Data for Lucidene

H	298 K		328 K <sup>a</sup>	
	$\delta$	multiplicity, $J$ (Hz)	$\delta$	multiplicity, $J$ (Hz)
6	2.56	d, 7.5 (2 H)		
7	5.63	ddd, 16, 7.5, 7.5		
8	5.73	d, 16		
10	1.55	d, 14, 1.5		
	1.08	dd, 14, 5.5		
10A	1.96	dddd, 11, 5.5, 5.5, 1.5		
11 $\alpha$	2.56	dd, 16, 11		
11 $\beta$	2.79	dd, 16, 5.5		
17	1.69	br m	1.72	ddd, 13, 13, 3.5
	1.81	br m	1.88	ddd, 13, 13, 3.5
18	1.81	br m	1.82	dddd, 12, 12, 4.5, 4.5
	2.08	v br s	2.05	br m,
18A	2.22	m	2.22	dddd, 13.5, 4.5, 4.5, 4.5
19 $\alpha$	2.79	br d	2.79	dd, 16, 13.5
19 $\beta$	2.67	br d	2.69	dd, 16, 4.5
5A-Me	1.25	s		
16A-Me	1.25	s		
9-Me	1.08	s		
	1.12	s		
Ar-H's	7.04	t, 7.5 (2 H)		
	7.03	d, 7.5		
	6.97	d, 7.5		
	6.80	t, 7.5		
	6.74	t, 7.5		
	6.73	d, 7.5 (2H)		

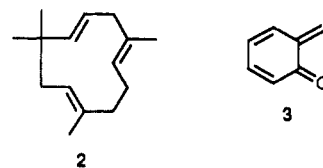
<sup>a</sup> Only signals which had significantly sharpened in comparison with the spectrum at 298 K were included.

Molecular models of the most likely conformations suggest that in one conformation the proton at C-19 is in the vicinity of the  $\pi$  system of the C-7, C-8 double bond, while in another this is not the case. Apparently, the anisotropic effect of the double bond is rather strong and responsible for the broadness of the signal at  $\delta$  2.08.

In the  $^{13}\text{C}$  NMR spectrum a similar phenomenon was observed. Six signals for aliphatic carbons were broad at 298 K and sharpened to a varying degree when the temperature was raised to 328 K. In addition, there was also a signal ( $\delta$  124.5) in the  $\text{sp}^2$  region which was very sharp at 328 K, but quite broad at 298 K. The  $^{13}\text{C}$  NMR spectrum showed only 13 signals in the  $\text{sp}^2$  region, instead of the expected 14. Apparently two signals have identical chemical shifts.

GC-MS analysis of the least polar chromatography fractions of the hexane extract of *U. lucida* ssp. *lucida* showed the presence of the known sesquiterpenes cyperene (39.3% of the combined nonpolar fractions, according to peak integration by GC),  $\beta$ -selinene (17.9%),  $\alpha$ -selinene (5.5%), humulene (<1%), and several other constituents, which were either present in very small concentration or unknown. Four unidentified sesquiterpenes (1.3%) showed the characteristic fragmentation pattern of sesquiterpenes with an oxybenzyl moiety ( $m/e$  310 and 107).

Lucidene (1) can be considered as an addition product of humulene (2), which was also present in the least polar fractions of the rootbark extract (vide supra). The observation that lucidene was isolated as a racemic mixture suggests a biosynthetic formation by a nonenzymatic pathway. In fact a conceivable formation of lucidene is a double Diels-Alder reaction of humulene with two molecules of *o*-benzoquinone methide (3). Apart from



reacting as a dienophile in Diels–Alder reactions, *o*-benzoquinone methide (**3**) can also undergo Michael conjugate addition reactions to produce *o*-hydroxybenzyl entities. Diels–Alder type products of terpenoids, as well as *o*-hydroxybenzyl-containing compounds, are well known among *Uvaria* metabolites.<sup>6,7,12</sup> The three dihydrochalcones isolated by us from the same plant, uvaretin, diuaretin, and chamuvaretin, are also metabolites containing *o*-hydroxybenzyl fragments.

The question whether other diastereomers (ring junction methyls trans) and regioisomers (oxygen of *o*-quinone methide bonded to less substituted carbon in cycloadduct) are formed as well cannot be answered here, since small quantities of such compounds may have remained undetected.

### Experimental Section

The melting points were determined on a "Electrothermal melting point apparatus", and are uncorrected. Mass spectra were taken with a Varian MAT 311a spectrometer and GC-MS with a VG MM7070F mass spectrometer using a 25 m CP-Sil 19CB column. <sup>1</sup>H NMR spectra were taken at 400 and 600 MHz (Bruker AM400 and AM600). All NMR spectra were taken in CDCl<sub>3</sub>, using TMS as an internal standard. Infrared spectra were taken with a Perkin-Elmer 257 spectrophotometer and ultraviolet spectra with a Unicam SP.800.

**Isolation Procedures.** The plant material used in this study was obtained from Chalinze, Tanzania, and identified by Mr. L. B. Mwasumbi, Department of Botany, University of Dar es Salaam. A voucher specimen was deposited at the Herbarium of the Department of Botany, University of Dar es Salaam.

The dried ground rootbark (385 g) of *U. lucida* ssp. *lucida* was extracted with petroleum ether (bp 60–80 °C) and fractionated by gravitational chromatography (silica gel) using a hexane/ethyl acetate gradient. The first fraction (9.4 g) contained mostly sesquiterpenes, and was analyzed by GC-MS. Subsequent fractions yielded, in order of elution: lucidene, benzyl benzoate, chamuvaretin, uvaretin, and diuaretin.

**Lucidene (5a,6,9,10,10a,16a,17,18,18a,19-Decahydro-4a,9,9,16a-tetramethyl-11H-cycloundeca[1,2-b:5,6-b']bis[1]-benzopyran).** Recrystallization from hexane yielded 55 mg of pure lucidene, as white crystals: mp 208–210 °C; UV λ<sub>max</sub> (*n*-hexane) 284 nm (ε 3.523 × 10<sup>3</sup>), 277 (ε 3.09 × 10<sup>3</sup>), 228 (ε 1.30 × 10<sup>4</sup>), and 220 (ε 1.52 × 10<sup>4</sup>); IR (KBr) 1610 and 1584 (C=C), 1487 and 1455 (C–H), 1262, 1246, 1235, and 1221 (C–O), and 760 and 753 cm<sup>-1</sup> (ortho-substituted phenyl group); mass spectrum (EI) *m/e* (rel abundance) 416 (M<sup>+</sup>, 35), 309 (M – CH<sub>2</sub>PhOH, 50), 198 (32), 171 (35), 159 (100), 158 (63), 145 (24), and 107 (PhOHCH<sub>2</sub><sup>+</sup>, 64); calculated for C<sub>29</sub>H<sub>36</sub>O<sub>2</sub> 416.2715, found 416.2720. The compound turned brown with anisaldehyde on TLC and was found to be a racemic mixture by X-ray analysis. <sup>1</sup>H NMR data are collected in Table I. <sup>13</sup>C NMR (CDCl<sub>3</sub>, 298 K, 100.6 MHz, signals indicated with an asterisk (\*) significantly sharpened when the temperature was raised to 328 K): 19.67, 21.34, 23.64\*, 26.60\*,

29.09, 29.69, 30.00, 31.77\*, 32.64, 35.75\*, 40.85\*, 46.07, 49.02\*, 79.46, 79.72, 116.66, 117.20, 119.30, 119.66, 121.69, 122.87, 124.50\*, 127.08, 127.31, 128.73, 129.15, 143.97, 153.53.

**Chamuvaretin.** The semipurified fraction containing chamuvaretin was further purified by column chromatography (fractogel, PVA 500), using acetone as the eluent, and by PTLC. Yield: 35 mg, MS, <sup>1</sup>H NMR, UV, and IR data were in agreement with those reported in the literature.<sup>12</sup> The compound turned brown with anisaldehyde on TLC.

**Diuaretin.** The semipurified fraction containing diuaretin was further purified by reverse-phase column chromatography (RP-2, H<sub>2</sub>O/acetone gradient) by fractogel chromatography (acetone) and by RP-PTLC (acetone/H<sub>2</sub>O). Weight: 36 mg. MS, <sup>1</sup>H NMR, UV, and IR data were in agreement with those reported in the literature.<sup>3</sup> The compound turned pink with anisaldehyde on TLC.

**Uvaretin.** The semipurified fraction containing uvaretin was further purified by reverse-phase column chromatography (RP-2, H<sub>2</sub>O/acetone gradient) and recrystallized from benzene. Weight: 30 mg; mp 161–163 °C (lit.<sup>3</sup> mp 164–165 °C). MS, <sup>1</sup>H NMR, UV, and IR were in agreement with those reported in the literature,<sup>3</sup> and the compound turned pink with anisaldehyde on TLC.

**X-ray Diffraction Analysis of Lucidene.** The crystal structure of **1** was determined by X-ray diffraction. Crystal data: triclinic, space group P $\bar{1}$ ; *a* = 9.417 (5) Å, *b* = 15.899 (9) Å, *c* = 17.011 (8) Å, α = 69.96 (4)°, β = 89.73 (5)°, γ = 82.015 (5)°; *V* = 2367 Å<sup>3</sup>; *Z* = 4; *d*<sub>calc</sub> = 1.17 g cm<sup>-3</sup>, μ = 0.66 cm<sup>-1</sup>. Reflections were measured in the Ω/2θ scan mode, using graphite monochromated Mo Kα radiation (scan width (Ω) 1.30 + 0.34 tan θ). The structure was solved by direct methods and refined with full-matrix least-square methods. A total of 2691 reflections with *F*<sub>o</sub><sup>2</sup> > 3σ(*F*<sub>o</sub><sup>2</sup>) was used in the refinement. The number of parameters refined was 435 (scale factor, positional and anisotropic thermal parameters for non H atoms, H atoms were treated as riding on their parent C atoms with fixed thermal parameters). The final *R* factors were *R* = 5.6% and *R*<sub>w</sub> = 5.9%. All calculations were done with SDP.<sup>14</sup> The unit cell contains two independent molecules with approximately the same conformation, one of which is shown in Figure 1.

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**Registry No.** 1, 128216-79-5; uvaretin, 58449-06-2; diuaretin, 61463-04-5; chamuvaretin, 64675-27-0.

**Supplementary Material Available:** Tables of positional and thermal parameters and bond distances and angles (11 pages). Ordering information is given on any current masthead page.

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