μL of a solution of myosin (10 mg/mL in 50% glycerol). The course of the reaction was followed by HPLC. After reaction for 60 and 120 min at 37 °C, a further 100 μL of myosin was added. After a 3-h incubation, 54% of the starting material had disappeared and a product with a HPLC retention time of 6.12 min had been formed. The reaction mixture was chromatographed as described above for the glycerol kinase reaction; yield of pure (S_pR_p)-GTPαSβS 125 A_{252} units (46%). The material was identical by ^{31}P NMR spectroscopy with that obtained by the enzymatic phosphorylation of (S_p)-GDPαSβS with acetate kinase.

(R_pR_p)-Guanosine 5'-O-(1,2-Dithiotriphosphate) (GTPαSβS). A mixture of (R_p,S_p)- and (R_pR_p)-GTPαSβS was reacted with myosine as described above. In this case the myosin-catalyzed hydrolysis was slow. Repeated additions of enzyme and longer reaction time (6 h) resulted only in a partial removal of the R_pS_p isomer. After DEAE-Sephadex purification, 8.7 μmol of a 3:1 mixture of (R_pR_p-R_pS_p)-GTPαSβS was obtained as analyzed by ^{31}P NMR spectroscopy.

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Tanzanene, a Spiro Benzopyranyl Sesquiterpene from *Uvaria tanzaniae* Verdc.

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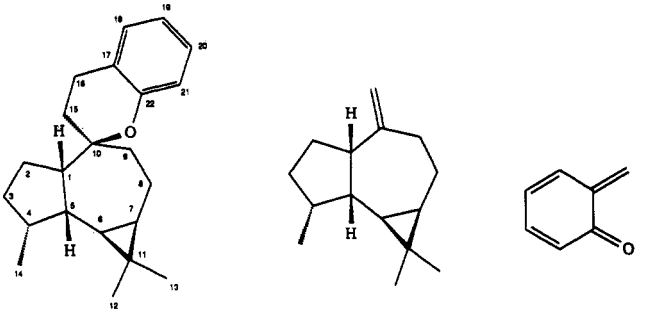
Tanzanene (1), a spiro benzopyranyl sesquiterpene, was isolated from the rootbark of *Uvaria tanzaniae* Verdc. and its structure was determined by high-resolution NMR. The molecular structure of tanzanene can be thought of as a cycloaddition product of alloaromadendrene (2) and the quinone methide of *o*-hydroxytoluene (6-methylene-2,4-cyclohexadien-1-one, 3). The known dihydrochalcones uvaretin, diuvaretin, chamuvaretin, and isotriuvaretin were also isolated from the same plant.

Introduction

As part of our search for new compounds with antimalarial activity from plants, we are investigating the genus *Uvaria*. The crude petroleum ether extract of the rootbark of *Uvaria tanzaniae* showed considerable activity against the multidrug resistant K_1 strain of *Plasmodium falciparum*[1] in vitro and was therefore studied in more detail. This led to the isolation of a new spiro benzopyranyl sesquiterpene, which we have named tanzanene (1), as well as the known dihydrochalcones uvaretin,[2] diuvaretin,[2] chamuvaretin,[2] and isotriuvaretin.[3] Tanzanene (1) has some features in common with other C-benzylated sesquiterpenes that have recently been isolated from *U. angolensis*[4] and *U. lucida* ssp. *lucida*.[5] However, unlike any other C-benzylated natural products reported so far, the C-benzyl substituent in 1 forms a spiro connection with the sesquiterpene part of the molecule.

Results and Discussion

Tanzanene (1) was isolated by silica gel chromatography of the petroleum ether extract of the rootbark of *Uvaria tanzaniae* using a gradient of hexane and ethyl acetate. The compound was obtained as white needles from methanol, mp 84-85 °C, $[\alpha]_D$ -4.5° (*c* 0.47, $CHCl_3$). The mass spectrum suggested an oxybenzylated sesquiterpene structure for 1, with characteristic fragments at *m/z* 310 (M^+, $C_{22}H_{30}O$), 203 ([M - $HOPhCH_2$]$^+$), 189 ([M - $HOPhCH_2CH_2$]$^+$), and 107 ([$HOPhCH_2$]$^+$). The UV (λ_{max} (EtOH) 284, 277, 225, 218 nm) and IR spectra (KBr, 1608, 1580 (C=C), 1247 (C—O), 746 cm^{-1} (ortho-substituted phenyl)) are in agreement with an *o*-oxybenzyl group in 1.[4,5]



1 2 3

The ^1H NMR spectrum of 1 indicated the presence of an alloaromadendrene (2) skeleton for the sesquiterpene

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Table I. ^{1}H NMR Spectral Data for Tanzanene (1), Ledol (4), and Viridiflorol (5)

H	tanzanene δ multiplicity; J (Hz)	ledol δ multiplicity; J (Hz)	viridiflorol δ multiplicity; J (Hz)
H-1	2.02 dddd; 9, 3.5, ?, 1.5	1.82 m	1.68 dddd; 13, 7, 3.5, 2
H-2	~1.4–1.5	2.02 m	1.54 dddd; 13, 7, 3.5, 1.5
H-2		1.30 m	1.45 dddd;, 13, 13, 11.5, 6
H-3	1.22 m	~1.7 (2 H)	1.73 dddd; 13, 9, 9, 6
H-3	~1.7[a]		1.22 dddd; 13, 11, 9, 4
H-4	1.71 m	1.85 m	1.88 m
H-5	2.06 m	1.52 ddd; 10, 6, 6	1.84 m
H-6	0.08 dd; 10, 9.5	0.17 dd; 10, 9.5	0.03 dd; 10, 9.5
H-7	0.59 ddd; 11.5, 9.5, 6.5	0.53 ddd; 11.5, 9.5, 6.5	0.55 m
H-8	1.84 dddd; 14, 11.5, 11.5, 1	1.03 dddd; 14.5, 11, 11, 5.5	
H-8	1.62 dddd; 14, 7, 7, 1	1.71 dddd; 14, 6, 6, 4.5	1.42 (1 H)
H-9	1.77 dddd; 14, 7, 1.5, 1	1.54 ddd; 14, 5, 5	1.5–1.65 (3 H)
H-9	1.43 ddd; 14, 11.5, 1	1.83 ddd; 14, 11, 5.5	
CH_3-12	1.07 s	0.87[b] s	1.03[b] s
CH_3-13	1.15 s	0.91[b] s	1.04[b] s
CH_3-14	0.92 d; 6.5	0.92 d; 7	0.96 d; 6.5
H-15	1.57 ddd; 13.5, 6.5, 6.5	1.16 s (CH_3)	1.04[b] s (CH_3)
H-15	1.45 ddd; 13.5, 6.5, 6.5		
H-16	2.50 ddd; 16, 6.5, 6.5		
H-16	2.44 ddd; 16, 6.5, 6.5		
H-18	6.97 d_{br}; 8		
H-19	6.81 ddd; 8, 8, 1.5		
H-20	7.05 dd_{br}; 8,8		
H-21	6.99 dd; 8, 1.5		

[a] Signal overlapped. [b] Assignments tentative.

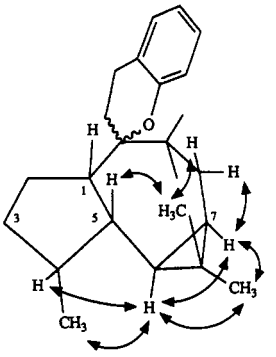
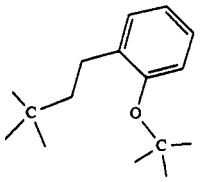


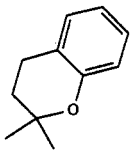
Figure 1. NOE enhancements observed for tanzanene.

moiety,[6,7] as well as an *o*-oxybenzyl group (see Table I). The ^{13}C NMR spectrum showed, in addition to signals for the *o*-oxybenzyl and sesquiterpene moieties, a peak at δ 79.25, indicating that the *o*-oxybenzyl moiety must be integrated into the molecule by an ether linkage, since the molecule contains only one oxygen atom.

^{1}H NMR decoupling experiments gave evidence that the *o*-oxybenzyl group is linked to the rest of the molecule as a benzopyran moiety in a spiro fashion. When decoupling the benzyl protons (H-16) (center at δ 2.47), the only alteration was the appearance of a clear AB system with signals at δ 1.57 and 1.45 (J_{AB} = 13.5 Hz). From this we deduce partial structure a, since the carbon atom neighboring the homobenzylic CH_2, as well as the carbon adjacent to the oxygen of the ether linkage, should be quarternary.

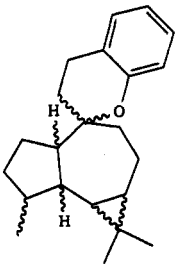


a



b

Long-range ^{13}C/^{1}H correlation demonstrated the identity of the aforementioned quarternary C atoms and, thereby, the spiro structure b. Further decoupling experiments revealed structural elements that are in agreement with an alloaromadendrene (2) skeleton,[6,7] having a quarternary C-10 and no exocyclic double bond. Therefore, the benzopyran moiety has to be linked to an alloaromadendrene-type skeleton as in c.



c

The C–H correlation spectra corroborated structure c; in particular, they demonstrated long-range couplings between C-10 and H-1 and H-5 and H-9, in addition to the couplings already mentioned for C-10 with H-15 and H-16. Difference NOE experiments established the relative configurations of protons and methyl groups in the C-3–C-8 region according to Figure 1.

The cis-fused ring system in tanzanene was demonstrated by decoupling experiments as well as by a phase-sensitive COSY study, which showed the coupling constant between H-1 and H-5 to be 3.5 Hz, the same value as measured for viridiflorol (5), whereas in the corresponding trans-fused system of spathulenol,[8] the coupling constant between these bridgehead protons is about 9 ppm.[8,9]

The ^{13}C data of ledol (4) and viridiflorol (5) (Table II), which are epimers at C-10,[10] exhibit small but characteristic differences of carbon resonances with the largest Δδ values (>3 ppm) for C-1 and C-7. Furthermore, Δδ be-

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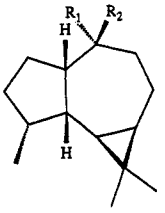
Table II. ^{13}C NMR Spectral Data for Tanzanene (1), Ledol (4), and Viridiflorol (5)[a]

C atom	1	4	5
C-1	53.67 (d)	54.30	58.56 (d)
C-2	24.56 (t)	25.03	26.03 (t)
C-3	29.06 (t)	31.12	29.52 (t)
C-4	38.40 (t)	38.78	38.77 (t)
C-5	39.93 (d)	41.03	40.09 (d)
C-6	22.78 (d)	23.74	22.75 (d)
C-7	28.78 (d)	25.33	29.07 (d)
C-8	18.35 (t)	20.70	19.22 (t)
C-9	33.66 (t)	39.63	38.02 (t)
C-10	79.25 (s)	73.98	73.92 (s)
C-11	19.15 (s)	19.02	18.60 (s)
C-12	16.40 (q)[b]	15.64[b]	16.44 (q)[b]
C-13	29.09 (q)	28.65	28.87 (q)
C-14	16.46 (q)[b]	16.32[b]	16.65 (q)[b]
C-15	32.69 (t)	31.20	32.46 (q)
C-16	22.17 (t)		
C-17	121.58 (s)		
C-18	129.67 (d)		
C-19	119.86 (d)		
C-20	127.88 (d)		
C-21	117.80 (d)		
C-22	154.11 (s)		

[a] Multiplicities by DEPT; assignments were based on C–H correlation spectra. [b] Assignments tentative.

tween the resonances of CH_3-12 and CH_3-14 is significantly larger for 4 than for 5.

When the standard increments caused by alkylation of CH_3-15 and by phenyl ether formation at the OH group (i, +9.6; α, −5.8; β, −1.0) were calculated for 4 and 5, the ^{13}C resonances measured for 1 fit well only to the values calculated on the basis of viridiflorol (5). This established the relative configuration at C-10 of tanzanene as depicted in 1.



4: R_1 = OH; R_2 = Me

5: R_1 = Me; R_2 = OH

GC and GC–MS analysis of the apolar sesquiterpene fraction of the roots of *U. tanzaniae* indicated that alloaromadendrene is one of the major sesquiterpenes present (19.7% of all apolar sesquiterpenes present). This observation supports the hypothesis that tanzanene (1) biogenetically might originate from alloaromadendrene (2) and 6-methylene-2,4-cyclohexadien-1-one (3), similar to a 2 + 4 cycloaddition reaction. Likewise, in *U. lucida* ssp. *lucida* we have detected the presence of both humulene and a possible 4 + 2 cycloaddition product.[5]

In addition, we have isolated the known C-benzylated dihydrochalcones uvaretin,[2] diuvaretin,[2] chamuvaretin,[2] and isotriuvaretin[3] from the same plant. Antimalarial testing[1] (growth inhibition of *Plasmodium falciparum* in vitro) showed activity for uvaretin (IC_{50}, 3.49 μg/mL), diuvaretin (IC_{50}, 4.20 μg/mL), chamuvaretin (IC_{50}, 8.31 μg/mL), and isotriuvaretin (IC_{50}, 20.85 μg/mL), but not for tanzanene (IC_{50}, ≥50 μg/mL).

Experimental Section

Plant Material. *Uvaria tanzaniae* was collected from the Lunguza forest, Tanga region in Tanzania. A voucher specimen has been deposited in the herbarium of the Botany Department, University of Dar es Salaam, Tanzania.

Isolation of Tanzanene and the Dihydrochalcones. The petroleum ether extract of the rootbark of *U. tanzaniae* (200 g) was fractionated by silica gel chromatography using petroleum ether/ethyl acetate gradient elution. The fraction containing tanzanene was purified by recrystallization from methanol to afford white needles of 1 (114 mg): mp 84–85 °C; $[\alpha]_D$ −4.5° (c 0.47, $CHCl_3$); UV λ_{max} (EtOH) 284, 277, 225 and 218 nm; IR ν_{max} (KBr) 2989, 2955, 2932, 2881, 2866, 1608, 1580, 1494, 1485, 1453, 1247, 1107, 746 cm^{-1}; all NMR spectra were taken in C_6D_6; ^1H NMR, see Table I; ^{13}C NMR see Table II; MS *m/z* (rel intensity) 310 (M^+, 98), 267 (28), 241 (17), 215 (11), 204 (13), 203 (79), 190 (27), 189 (66), 147 (56), 133 (27), 121 (34), 107 (100), 105 (30), 95 (30), 93 (24), 91 (41), 81 (30), 69 (40); calcd for $C_{22}H_{30}O$ 310.2297, found 310.2295.

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Registry No. 1, 135074-06-5.

Supplementary Material Available: ^1H-decoupled ^{13}C NMR spectrum of tanzanene (1 page). Ordering information is given on any current masthead page.