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SESQUITERPENOIDS FROM *BRACHYLAENA HUTCHINSII*

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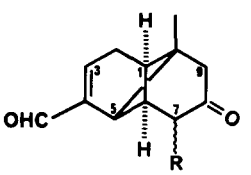
ABSTRACT.—The MeOH extract from the bark of *Brachylaena hutchinsii* yielded two ketoaldehyde sesquiterpenes, 8-ketocopaenal [**1**] and 8-ketoylangenal [**2**], which are also known as brachylaenalones A and B, respectively. In addition to the above-mentioned aldehydes, the corresponding ketoalcohols 8-ketocopaenol [**3**] and 8-ketoylangenol [**4**] were also isolated from the same plant, along with a fifth non-ketonic compound, ylangenol [**5**]. Although the ketoaldehydes **1** and **2** have already been described in the literature, their structures have not been completely established. We describe the isolation, identification, and antibacterial activity of five sesquiterpenes from *Bra. hutchinsii* and propose new structures for brachylaenalones A and B based on their spectral data.

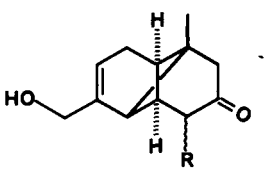
The isolation of sesquiterpenes, mainly cadinene and copaene derivatives, from *Brachylaena hutchinsii* Hutch. (Compositae) has been reported previously (1,2). In our continuing search for biologically active natural compounds, we have found that the MeOH extract from *Bra. hutchinsii* has antibacterial activity against Gram positive bacteria, particularly *Streptococcus mutans* and *Brevibacterium ammoniagenes*. Further bioassay-guided fractionation led to the isolation of three active principles **1**, **2**, and **5**, as well as two similar congeners **3** and **4**. Among the active compounds, compound **5** showed the strongest antibacterial activity. An analysis of ^{13}C- and ^{1}H-nmr spectra proved to be important in determining the correct structures for these compounds. Compounds **1** and **2** were identified as brachylaenalones A and B, which have previously been isolated from the same species. Based on spectroscopic evidence we propose revised structures **1** and **2** for these compounds. Although compound **5** was previously isolated from a composite, *Wunderlichia mirabilis* (3), this is the first report of its antimicrobial activity.

RESULTS AND DISCUSSION

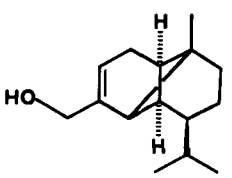
The MeOH extract of the bark of *Bra. hutchinsii* afforded, after various chromatographies (see Experimental), five sesquiterpenes **1**–**5**. Compounds **1** and **2** were identical with the previously reported brachylaenalones A and B (2). Their mass spectra showed



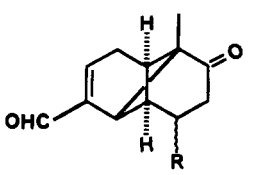
1 R=α-iPr
2 R=β-iPr



3 R=α-iPr
4 R=β-iPr



5



6 R=α-iPr
7 R=β-iPr

identical molecular ions at *m/z* 232 and identical fragmentation patterns. These data in conjunction with ^{1}H- and ^{13}C-nmr data allowed us to propose the molecular formula $C_{15}H_{20}O_2$ for these two compounds. The ^{1}H-nmr spectra obtained for both compounds showed signals corresponding to three methyls, one at δ 0.75 ± 0.01 due to a quaternary methyl group, and two other signals at δ 0.95 and δ 0.80 ± 0.02 indicative of isopropyl methyls. Two signals at low field δ 9.50 ± 0.02 and δ 6.79 ± 0.01 were attributed to an aldehyde and a vinylic proton, respectively. Based on ^{1}H-^{1}H and ^{1}H-^{13}C COSY experiments, it was possible to completely assign all of the ^{1}H and ^{13}C signals for these molecules (Tables 1 and 2). From the data described above we could propose sesquiterpenoid structures for **1** and **2**. However, these structures are different from those described previously (2). This conclusion was based principally on nOe experiments, where a strong correlation between H_2-9 and Me-14 was sufficient to rule out the previous structures **6** and **7**. The revised structures also explain why these compounds could not be transformed into the corresponding α,β-unsaturated ketones when oxidation experiments were carried out (2); the α,β-unsaturated ketones could not be obtained without opening one of the rings in this tricyclic system.

TABLE 1. ^{1}H nmr of the Sesquiterpenes **1–5** (500 MHz, $CDCl_3$, δ scale).[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1	2.05 m	2.14 m	2.08 m	1.99 m	
H-2α	2.73 dt	2.69 dt	2.39 dt	2.45 dt	2.25 m
H-2β	2.61 dt	2.61 dt	2.31 dt	2.34 dt	2.25 m
H-3	6.79 brt	6.78 brt	5.64 s	5.61	
H-5	2.89 d	2.79 d	2.05	2.15 d	
H-6	1.86 d	1.89 d	1.99 d	2.00 d	
H-7	2.25 brd	2.37 brd	2.31	2.25 dd	
H-9α	2.55 d	2.58 d	2.50 d	2.47 s	
H-9β	2.48 d	2.46 d	2.45 d	2.47 s	
H-11	2.30 m	2.35 m	2.29 m	2.32 m	
H-12	0.82 d	0.79 d	0.80 d	0.83 d	0.81 d
H-13	0.95 d	0.95 d	0.96 d	0.95 d	0.83 d
H-14	0.75 s	0.76 s	0.84 d	0.84 d	0.77 s
H-15	9.51 s	9.48 s	4.02 s	4.00 s	3.96 s

[a]Coupling constants for compound **1–4**: $J_{1,5} = 6.4$; $J_{2\alpha,2\beta} = 20.5$; $J_{2\alpha,3} = J_{2\beta,3} = 3.2$; $J_{6,7} = 2.3$; $J_{9\alpha,9\beta} = 18.5$; $J_{11,12} = 6.7$.

An analysis of the ^{13}C-nmr chemical shifts of C-1 and C-5 revealed that compounds **1** and **2** are epimeric at C-7. The chemical shifts of these carbons are diagnostic (4) of the stereochemistry of the isopropyl group. For example, in compound **1**, the chemical shift for C-1 should appear at lower field while for C-5, the chemical shift should appear at higher field, with respect to the corresponding carbons in compound **2**. The complete ^{13}C-nmr assignments for these sesquiterpenes can be found in Table 2.

We envisioned that compounds **1** and **2** are interconvertible. Isomerization of compound **1** in basic solution gave a mixture of **1** and **2**, but treatment with acidic MeOH did not cause isomerization because the aldehyde group was protected as its hemiketal (or ketal) derivative.

The ^{1}H- and ^{13}C-nmr data obtained for compounds **3** and **4** are very similar to those obtained for **1** and **2**. Again a sesquiterpenoid skeleton was proposed for these compounds, where the only difference was the stereochemistry of the isopropyl group. From

TABLE 2. ^{13}C nmr of the Sesquiterpenes **1–5** (125 MHz, $CDCl_3$, δ scale).

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
C-1	41.93	45.06	41.98	45.51	44.47
C-2	31.55	31.77	29.85	30.09	29.90
C-3	146.71	146.67	118.37	118.87	117.90
C-4	149.21	148.89	145.57	145.28	147.26
C-5	42.23	38.65	47.33	44.00	44.50
C-6	40.41	40.70	40.97	40.93	37.28
C-7	58.56	59.35	59.06	59.66	50.36
C-8	211.21	211.47	212.45	212.39	21.67
C-9	51.86	51.90	52.31	52.38	35.98
C-10	38.09	38.00	38.49	38.37	39.31
C-11	27.98	28.25	28.05	28.11	32.14
C-12	19.23	19.41	19.35	19.26	19.92
C-13	21.14	21.09	21.12	21.14	19.63
C-14	18.58	18.56	18.79	18.72	19.53
C-15	190.72	190.62	65.64	65.77	65.97

the nmr data it was possible to conclude that **3** and **4** are the corresponding alcohols of aldehydes **1** and **2** (^1H δ 4.01 ± 0.05 2H, ^{13}C δ 65.70 ± 0.07). This is in perfect agreement with the mass spectrum, which showed a molecular ion at *m/z* 234, corresponding to a molecular formula of $C_{15}H_{22}O_2$. The spectral data obtained for these two compounds are very closely related to those described for α-copaen-8-one isolated from *Neomirandea guevarii* (5). The only difference between the latter compound and compounds **3** and **4** is a 15-methyl instead of a 15-hydroxymethylene group.

A fifth sesquiterpene, ylangenol [**5**], isolated from the same plant had spectroscopic features similar to those of the four described above. The only difference observed in the ^{13}C-nmr spectrum was the absence of a ketonic carbonyl and the presence of a methylene group at 21.67 ppm. This observation was reinforced by the mass spectrum with a molecular ion *m/z* 220. This compound also contains a primary alcohol represented by the signals ^1H δ 3.96 and ^{13}C δ 65.97. These data led to the structure of the sesquiterpene, ylangenol, as **5**. A comparison of the ^{13}C chemical shifts of **5** with those of related compounds isolated from this plant allowed us to propose a ylangenol stereochemistry for this compound rather than the stereochemistry of its isomer, copaenol. However, the ^{13}C chemical shifts observed for **5** are very similar to those reported for 15-copaenol (6).

The preliminary antimicrobial assay against four typical microorganisms, *Bacillus subtilis, Escherichia coli, Saccharomyces cerevisiae*, and *Penicillium chrysogenum*, indicated that the MeOH extract of the barks of *Bra. hutchinsii* had activity against only *Ba. subtilis*, similar to many other plant extracts (7). A more detailed bioassay with four additional Gram positive bacteria revealed that *Str. mutans* and *Bre. ammoniagenes* were among the most sensitive. The five isolated compounds were then subjected to the final assay, which was carried out at the highest concentration of 800 μg/ml because of limited solubility and availability of the samples, and the results are summarized in Table 3. From these data it is possible to conclude that ylangenol [**5**], a compound containing a methylene instead of carbonyl at C-8, has the strongest antibacterial activity. Comparison of the biological activities of the aldehydes **1** and **2** with those of the corresponding alcohols **3** and **4**, even though the activity was weak, indicated that the aldehydes presented stronger activity. It has been suggested that the presence of an α,β-unsaturated carbonyl may be responsible for the activity in this type of structure.

TABLE 3. Minimal Inhibitory Concentration for Compounds **1–5** Against *Streptococcus mutans* and *Brevibacterium ammoniagenes* (μg/ml).

Compound	*Str. mutans*	*Bre. ammoniagenes*
1	800	800
2	800	800
3	>800	>800
4	>800	>800
5	25	25

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were acquired on a Perkin-Elmer 1310 ir. Eims spectra were taken on a JEOL DX-303HF. Nmr spectra were recorded on a JEOL GSX-500 (500 MHz for ^1H and 125 MHz for ^{13}C). Recycling hplc was performed on a JAI LC-09 (Japan Analytical Industry, Tokyo, Japan).

PLANT MATERIAL.—The barks of *Bra. hutchinsii* were collected near Nairobi, Kenya and identified by the East African Herbarium, Nairobi, Kenya where a voucher specimen was deposited.

EXTRACTION AND ISOLATION.—The powdered plant material was extracted with MeOH at ambient temperature. The solvent was removed under reduced pressure at 40° to yield a dark brown residue (400 g). A portion of this residue (2 g) was chromatographed over a Si gel (100 g) column using as eluent CH_2Cl_2, EtOAc, and MeOH, pure and in mixture, in order of increasing polarity. The CH_2Cl_2 extract exhibited antibacterial activity. All brachylaenalones were found in the fractions eluted with CH_2Cl_2. The fraction containing a mixture of brachylaenalones A and B was chromatographed over Si gel (Lichroprep) using *n*-hexane–EtOAc (9:1) as eluent to yield pure brachylaenalone A (160 mg) and a mixture of brachylaenalones A and B. This mixture was then purified by using recycling hplc, yielding brachylaenalone A (10 mg) and brachylaenalone B (60 mg). Fractions containing the remaining compounds were purified by low pressure cc to afford compounds 8-ketocopaenol [**3**] (60 mg), 8-ketoylangenol [**4**] (50 mg), and ylangenol [**5**] (15 mg).

8-Ketocopaenal [**1**].—Colorless oil: ir (film) 1715, 1680, 1625 cm^{-1}; eims *m/z* (rel. int.) 232 (20), 190 (60), 161 (50), 133 (70), 91 (100).

8-Ketoylangenal [**2**].—Colorless oil: ir (film) 1715, 1680, 1625 cm^{-1}; eims *m/z* (rel. int.) 232 (30), 190 (50), 161 (40), 133 (80), 91 (100).

8-Ketocopaenol [**3**].—Colorless oil: ir (film) 3400, 1715 cm^{-1}; eims *m/z* (rel. int.) 234 (40), 216 (85), 135 (80), 91 (100).

8-Ketoylangenol [**4**].—Colorless oil: ir (film) 3400, 1715 cm^{-1}; eims *m/z* (rel. int.) 234 (100), 217 (92), 199 (60), 91 (50).

Ylangenol [**5**].—Colorless oil: ir (film) 3400 cm^{-1}; eims *m/z* (rel. int.) 220 (100), 202 (10), 177 (50), 135 (40).

ISOMARIZATION OF COMPOUND **1** (BRACHYLAENALONE A).—Compound **1** (1 mg) in MeOH (1 ml) was stirred with a drop of 1 M KOH in MeOH for 20 min at room temperature. The resulting mixture was neutralized with HOAc and extracted with CH_2Cl_2. Analysis of the CH_2Cl_2 extract by tlc revealed the presence of a mixture of **1** and **2** (brachyaenalones A and B).

ANTIMICROBIAL ASSAY.—*Test microorganisms.*—Twelve microorganisms were utilized as test organisms, selected on the basis of their varying characteristics. All microorganisms for the antimicrobial assay were purchased from American Type Culture Collection (Rockville, MD): *Ba. subtilis* ATCC 9372, *Bre. ammoniagenes* ATCC 6872, *Propionibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 12598, *Str. mutans* ATCC 25175, *Es. coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC 10145, *Enterobacter aerogenes* ATCC 13048, *Sa. cerevisiae* ATCC 7754, *Candida utilis* ATCC 9226, *Pityrosporum ovale* 14521, and *Pe. chrysogenum* ATCC 10106.

Media.—A mixture of 0.8% nutrient broth (BBL), 0.5% yeast extract (DIFCO), and 0.1% glucose was used for the culture of bacteria except *Str. mutans*. Brain heart infusion broth (3.7%) (DIFCO) was utilized for the culture of *Str. mutans*. Malt extract broth (2.5%) was used for the culture of fungi except *Pi.*

ovale. A mixture of 1% bactopeptone, 0.5% yeast extract, 1% glucose, and 0.1% corn oil was utilized for the culture of *Pi. ovale*.

Ba. subtilis, *Sa. cerevisiae*, *C. utilis*, *Pi. ovale*, and *Pe. chrysogenum* were cultured with shaking at 30°. *Bre. ammoniagenes* and *En. aerogenes* were cultured at 30° without shaking, and other microorganisms were cultured at 37° without shaking.

Minimal inhibition concentration (MIC).—The minimal inhibitory concentration was measured by the twofold serial broth dilution method (8). Microorganisms were cultured in a broth medium which contained a series of tubes with different concentrations of the test compounds. For the antimicrobial assay, all microorganisms were cultured without shaking except *Pe. chrysogenum*, which was cultured with shaking. After 48 h (5 days for *Pe. chrysogenum*), the growth of microorganisms was examined as turbidity (O.D. at 660 nm) except for *Pe. chrysogenum* and *Pi. ovale*, which were observed with the naked eye. The lowest concentration of the test compounds in which no growth occurred was defined as the MIC.

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