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SESQUITERPENES FROM THE ESSENTIAL OIL OF *CYPERUS ALOPECUROIDES*

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Key Word Index—*Cyperus alopecuroides*; norsesquiterpene; sesquiterpene hydrocarbons; cyprotene; 2,4-patchouladiene; 2,4,11-eudesmatriene; 3,5,11-eudesmatriene; epoxycyperene.

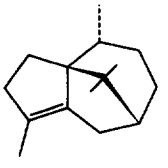
Abstract—The new norsesquiterpene hydrocarbon, cyprotene, the sesquiterpenes 2,4-patchouladiene, epoxycyperene, 2,4,11-eudesmatriene and 3,5,11-eudesmatriene, were identified as constituents in the hydrodistillation products of *Cyperus alopecuroides* from Cameroon by spectroscopic methods and chemical conversions.

INTRODUCTION

The essential oils of *Cyperus* species have long been used in folk medicine and as raw material for perfumes. In addition, plant growth regulating activities are associated with *Cyperus* species [1–3]. As major sesquiterpene hydrocarbon constituents cyperene (**1**) [4] and rotundene (**2**) [5] have been identified, while α-cyperone (**3**) [6] and cyperotundone (**4**) [7] are found as the main oxygenated compounds in most *Cyperus* species. Some minor constituents were also described in several reports [1, 8]. Our investigations of the hydrodistillation product of the rhizomes of *C. alopecuroides* led us to the isolation of five new sesquiterpenoids. The present communication deals with the structure determination of a cyperene related norsesquiterpene, two patchoulane-type and two eudesmane-type sesquiterpene compounds.

RESULTS AND DISCUSSION

GC-MS analysis of the essential oil of *C. alopecuroides* revealed the presence of several unknown sesquiterpenes. Repeated prep. GC [9] allowed the isolation of cyprotene (**5**), $C_{14}H_{24}$, $[M]^+ = m/z$ 192 (EIMS). The 14 carbon atoms were assigned by means of the ^{13}C NMR and DEPT spectra. These showed the presence of the olefinic (δ 115.7 and 145.6), carbons, five methyl, three methylene and three methine groups, and three quaternary carbon atoms. The ^1H NMR spectrum showed the presence of three tertiary methyl groups (δ 0.87, 0.90 and 1.04), one secondary methyl group (δ 0.83, *d*, $J = 6.61$ Hz), one olefinic methyl group (δ 1.72, *dt*, $J_1 = 6.61$ Hz and $J_2 = 1.54$ Hz), and an olefinic proton (δ 5.19, *qt*, $J_1 = 6.62$ Hz and $J_2 = 1.54$ Hz). The ^1H and ^{13}C NMR signal assignments, achieved by ^1H–^1H COSY and ^1H–^{13}C 2D correlation techniques, allowed the assumption of three substructures *a*, *b* and *c*. The olefinic proton at δ 5.13 is geminal to the methyl group at δ 1.72 and couples with CH_2-6 which again couples with the methine CH-5, leading to the substructure *a*: CH_3-CH=C(C)-CH_2-C(C)H-. The methine proton at δ 1.72 (vicinal to the methyl group at δ 0.83) couples with CH_2-3 which is connected to another methylene CH_2-4 which again couples with the methine CH-



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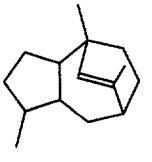

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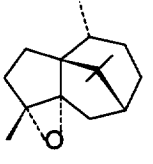

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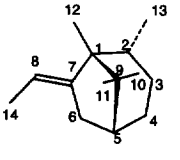

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* Author to whom correspondence should be addressed.

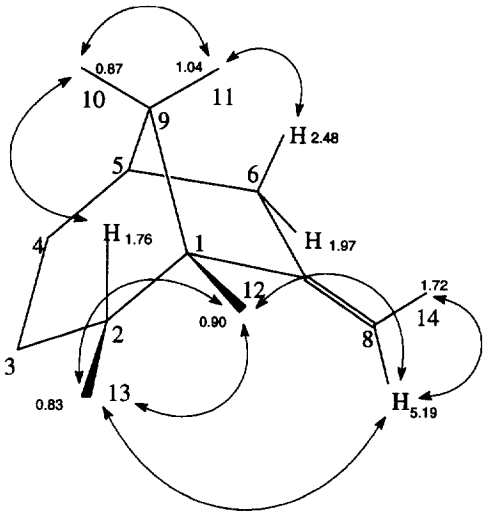

Fig. 1. Spatial correlations derived from NOESY spectra of **5**.

5, giving the substructure *b*: $CH_3CH(C)$-CH_2-CH_2-CH(C)-. The ^1H–^1H COSY spectrum shows a coupling correlation between the two methyl groups at δ 0.87 (CH_3-10) and 1.04 (CH_3-11), suggesting that they are geminal groups. Moreover, long-range coupling correlations were observed between these two methyl protons and the two carbon atoms (δ 43.1, C-5 and δ 51.1, C-1) giving the substructure *c*: $(CH_3)_2C(CH)$-C-CH_3. On combining this information with that of the ^1H–^{13}C long range connectivity diagram which shows that the methyl protons (δ 0.90, CH_3-12) were located three bonds from the olefinic quaternary carbon (δ 145.7, C-7) and also three bonds from the tertiary carbon atom (δ 34.7, C-2), structure **5** was arrived at for cyprotene. **5** has been isolated by us previously from the essential oil of *C. rotundus* [10]. The relative configuration was assigned by using two important sets of correlations of the NOESY spectrum (Fig. 1). The olefinic proton H-8 shows strong correlations to the C-13 methyl protons (δ 0.83) and also to the C-12 (δ 0.90) and C-14 (δ 1.72) methyl protons. Furthermore, the methine group proton H-2 (δ 1.76) is correlated to the C-10 and C-12 methyl protons δ 0.87 and δ 0.90. **5** is most likely a biogenetic degradation product or precursor of cyperene (**1**), the most abundant sesquiterpene hydrocarbon in most *Cyperus* species. Therefore, the absolute configuration of **1** and **5** is most probably identical.

The second unknown compound (**6**) was assigned a molecular formula of $C_{15}H_{22}$ ($[M]^+$, m/z = 202). The ^{13}C NMR and DEPT spectra permitted the identification of all the 15 carbon atoms: four methyl groups, three methylene groups, four methine groups, two of which are olefinic, and four quaternary carbon atoms, two of which are also olefinic. The ^1H NMR spectrum displays a cyperene type pattern, i.e. one secondary methyl group (δ 0.55, *d*, J = 6.61 Hz), two tertiary methyl groups (δ 0.51, *s* and δ 1.12, *s*) and an olefinic methyl group (δ 1.73, *s*). Of importance is the presence of two olefinic protons coupling with each other (δ 5.82, *d*, J = 5.08 Hz and δ 6.09, *d*, J = 5.08 Hz). The value of the coupling constant (J = 5.08 Hz) also suggests the presence of a five-membered ring substructure in the molecule. Thus, structure **6** was derived from the ^1H–^1H COSY and ^1H–^{13}C HMQC spectra. The ^1H–^1H COSY diagram shows a coupling correlation between the vinylic methyl group (δ 1.73 CH_3-15) and the methylene protons (δ 2.10, 1H, *d*, J = 17 Hz; δ 2.30, 1H, *dd*, J_1 = 17 Hz, J_2 = 2.30 Hz), which are themselves correlated to the methine proton CH-7 (δ 2.08, *m*), leading to the substructure $CH_3C(C)$=C(C)-CH_2-CH-. The methine proton CH-10 (δ 2.25, *m*, J = 6.1 Hz) couples with the methylene protons CH_2-9 (δ 1.27 Hz, *dd*, J_1 = 12.72 Hz, J_2 = 6.61 Hz; δ 1.68, *m*) which couples with another methylene CH_2-8 (δ 1.95, 1H, *tdd*, $J_1 = J_2$ = 12.72, J_3 = 6.62 Hz; δ 1.68, 1H, *m*) which is itself coupled to the methine proton CH-7 (δ 2.08, 1H, *m*), giving the substructure CH_3-CH(C)-CH_2-CH_2-CH-. On combining all this information, we arrived at structure **6** which proved to be in accord with the HMBC diagram for the ^1H–^{13}C long-range connectivities. The relative stereochemistry was assigned by means of the NOESY spectrum and by comparison with one of two products obtained by reduction and subsequent dehydration of cyperotundone (**4**) (Fig. 2). Besides **6**, isopatchoula-3,5-diene (**10**) was formed, which has been described in the literature as a constituent of *Cyperus scariosus* [11].

To the third compound (**7**), the molecular formula $C_{15}H_{24}O$ was assigned by EIMS ($[M]^+$, m/z = 220). The ^{13}C NMR spectrum allowed the identification of the 15 carbon atoms. The multiplicity was derived from the DEPT spectrum which shows four methyl groups, five methylene groups, two methine and four quaternary carbon atoms. That leads to an elemental composition of $C_{15}H_{24}$, which shows that the product is not an alcohol. Moreover, the ^{13}C NMR spectrum exhibits no carbonyl carbon. Hence, it was presumed that the product is an epoxide. The ^1H NMR spectrum displays patchoulane type patterns: one secondary methyl group (δ 1.04, *d*, J = 7.12 Hz), two tertiary methyl groups (δ 0.82, *s* and 0.91, *s*) and a fourth methyl group (δ 1.25, *s*). Because of the overlapping of the other proton signals, it was not possible to extract more information from the ^1H NMR spectrum. However, the fact that the compound was most likely an epoxide with a patchoulane type skeleton allowed the assumption that the compound is epoxycyperene (**7**). To verify this conclusion, we prepared epoxycyperene by epoxidation of cyperene (**1**). The product had identical spectroscopic data (MS and ^1H NMR) to the isolated compound.

Following our investigation of the sesquiterpene hydrocarbon fraction of the oil, we focused our attention on a subfraction which contained rotundene (**2**), β-selinene and, according to their mass spectra, two unknown minor compounds. Subsequent frac-

tionation by preparative GC afforded **8** as a pure product and a mixture of **9** and β-selinene.

To compound **8** the molecular formula $C_{15}H_{22}$ was assigned in accordance with its mass spectrum ($[M]^+$, $m/z = 202$). Because of the small quantity of the isolated product, not all of the carbon atom signals could be observed in the ^{13}C NMR spectrum. However, it was obvious that the compound contains three methyl groups, four methylene groups, one being olefinic (δ 110.83) and three methines (δ 44.82, 122.42 and 129.25). In the ^1H NMR spectrum, one observes two protons (δ 4.72, *m*, 2H) in the olefinic region coupling with the olefinic methyl group at δ 1.77 in the ^1H–^1H COSY diagram and suggesting the presence of an isopropenyl substituent in the molecule. The olefinic proton at δ 5.72, (1H, H-3, *dd*, $J_1 = 8.5$–9 Hz, $J_2 = 3.05$ Hz) couples with another olefinic proton at δ 5.66 (1H, H-2, *ddd*, $J_1 = 8.5$–9 Hz, $J_2 = 6.1$ Hz, $J_3 = 2.03$ Hz), which again couples to a methylene group at δ 1.96 (1H, *dd*, $J_1 \cong 17$ Hz, $J_2 = 6.1$ Hz) and δ 2.10 (1H, *d(br)*, $J \cong 17$ Hz), leading to the substructure C-CH=CH-CH_2-C. Moreover, the value of the coupling constant between the olefinic protons ($J = 8.5$–9 Hz) suggests that the substructure belongs to a six-membered ring. The ^1H–^1H COSY diagram shows that the olefinic methyl group (δ 1.71, *s(br)*) couples with a methylene group CH_2-6 (δ 1.86, *d(br)*, 1H, $J \cong 14.50$ Hz; δ 2.60, *dt(br)*, 1H, $J_1 \cong 14.50$ Hz, $J_2 \cong 3$ Hz), which itself couples with a methine proton CH-7 (δ 1.93, *dt(br)*, 1H, $J_1 = 6.61$ Hz, $J_2 \cong 3$ Hz), giving the substructure CH_3(C)C=C(C)-CH_2-CH(C)-. The angular methyl group (δ 0.98, *s(br)*) couples with the methylene CH_2-9 (δ 1.38, *dd*, 1H, $J_1 = 12.71$ Hz, $J_2 = 3.56$ Hz; δ 1.65–1.70, *m*) which itself couples with another methylene CH_2-8 (δ 1.48, *dd*, 1H, $J_1 = 12.71$ Hz, $J_2 = 3.56$ Hz; δ 1.65–1.70, *m*) which again couples to the methine proton CH-7, yielding the substructure CH_3-C-CH_2-CH_2-CH-. The combination of the four substructures affords structure **8**.

In order to confirm this structure, we reduced (+)-α-cyperone (**3**). Dehydration of the obtained alcohol led to two products which were identified as **8** and **9** (Fig. 2). The preparation of **9** from (+)-α-cyperone was very useful for its spectroscopic characterization since it could not be isolated as a pure product from the oil. The molecular formula $C_{15}H_{22}$ was deduced from the EIMS spectrum ($[M]^+$, $m/z = 202$). Except for three quaternary carbon signals, all the other signals appear in the ^{13}C NMR spectrum. The DEPT technique permitted the identification of three methyl groups, five methylene groups, one being olefinic and three methine, two of which are olefinic. The ^1H NMR spectrum also confirms the structure: two olefinic methyl groups (δ 1.78, CH_3-14, *m*; δ 1.73, CH_3-13, *s(br)*), one angular methyl group (δ 0.99, CH_3-15, *s*), four olefinic protons (δ 4.73, *m*, 1H, H-12; δ 4.77, *m*, 1H, H-12; δ 5.52, *m*, 1H, H-3; δ 5.41, *m*, 1H, H-2), one allylic methine proton (δ 2.91, *t(br)*, 1H, H-7, $J = 8.14$ Hz) and two allylic methylene protons CH_2-2 (δ 2.26, *m*; δ 2.05, *m*).

EXPERIMENTAL

Plant material. C. alopecuroides was collected in Kollbisson (Cameroon) in May 1995. A voucher specimen is deposited at the National Herbarium of Yaounde (Cameroon). The essential oil was obtained by hydrodistillation of the air dried rhizomes using *n*-hexane as collection solvent.

The fractionation of the oil was performed by prep. GC [9] on a Varian 2800 instrument, equipped with a stainless steel column (Silcosteal, Amchro) (2.05 m × 5.1 mm) with 6% octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin/polysiloxane PS-086 (1:1) on Chromosorb W-HP. He was used as carrier gas at a flow rate of 240 ml min^{-1}. A two ramp temp. programme was used: 120° during the first 25 min and 160° for the rest of the time. The preliminary sepn yielded 20 frs, the first six (CA_1 bis CA_6) being sesquiterpenoid hydrocarbon frs followed by oxygenated sesquiterpenoid frs. Further sepns and purifications were made using a stainless steel column with 2.5% (6-*O*-thexyl-2,3-di-*O*-methyl)β-CD (20% in polysiloxane SE 52), on Chromosorb G-HP (100–120 mesh) at 120°.

Cyprotene (**5**). The fr. CA_1 purified by prep. GC yielded a crystalline compound, mp 22–23°; ^1H NMR (400 MHz); δ 0.83 (3H, *d*, $J = 6.62$ Hz), 0.87 (3H, *s*), 0.90 (3H, *s*), 1.04 (3H, *s*), 1.03 (1H, *m*), 1.28 (1H, *m*), 1.38 (1H, *m*), 1.76 (1H, *m*), 1.89 (1H, *tddd*, $J_1 = J_2 = 13.22$ Hz, $J_3 = 6.23$ Hz, $J_4 = 2.54$ Hz, $J_5 = 1.14$ Hz), 1.97 (1H, *d(br)*, $J = 18$ Hz) 2.46 (1H, *d(br)*, $J = 18$ Hz), 5.19 (1H, *qt*, $J_1 = J_2 = J_3 = 6.61$ Hz, $J_4 = J_5 = 2.54$ Hz); ^{13}C NMR (100 MHz): 14.3, 15.4, 16.9, 18.8, 26.7, 28.8, 33.4, 34.7, 37.8, 43.1, 44.1, 51.1, 115.7, 145.7; MS (EI, 70 eV) *m/z* (rel. int.): 192(39) $[M]^+$ 177(67), 163(11), 149(33), 135(100), 121(78), 107(59), 93(31), 79(21), 67(20), 55(28), 41(56).

2,4-*Patchouladiene* (**6**). The sepn of fr. CA_2 yielded a colourless oil: ^1H NMR: δ 0.51 (3H, *s*), 0.55 (3H, *d*, $J = 6.61$ Hz), 1.12 (3H, *s*), 1.27 (1H, *d(br)*, $J_1 = 12.72$ Hz, $J_2 = 6.61$ Hz) 1.45 (1H, *m*), 1.68 (1H, *m*), 1.73 (3H, *s(br)*), 1.95 (1H, *tdd*, $J_1 = J_2 = 12.72$ Hz, $J_3 = 6.62$ Hz, $J_4 = 2.55$ Hz), 2.08 (1H, *m*), 2.10 (1H, *d(br)*, $J = 17$ Hz), 2.30 (1H, *dd(br)*, $J_1 = 17$ Hz, $J_2 = 6.62$ Hz), 5.82 (1H, *d*, $J = 5.08$ Hz), 6.09 (1H, *d*, $J = 5.08$ Hz); ^{13}C NMR (100 MHz): 13.57, 18.90, 21.75, 24.28, 28.86, 31.80, 73.90, 41.53, 51.06, 133.52, 137.68, 151.55; MS (EI, 70 eV) *m/z* (rel. int.): 202(32) $[M]^+$, 187(51), 177(10), 159(100), 145(37), 131(36), 119(54), 105(38), 91(36), 77(21), 69(13), 63(5), 55(20), 51(9).

The purification of the third fraction CA_3 gave the already known hydrocarbon cyperene (**1**). The separation of the fifth fraction CA_5 by prep. GC yielded 2,4,11-eudesmatriene (**8**), 3,5,11-eudesmatriene (**9**) and the known hydrocarbons rotundene (**2**) and β-selinene.

2,4,11-*Eudesmatriene* (**8**). ^1H NMR (400 MHz): δ

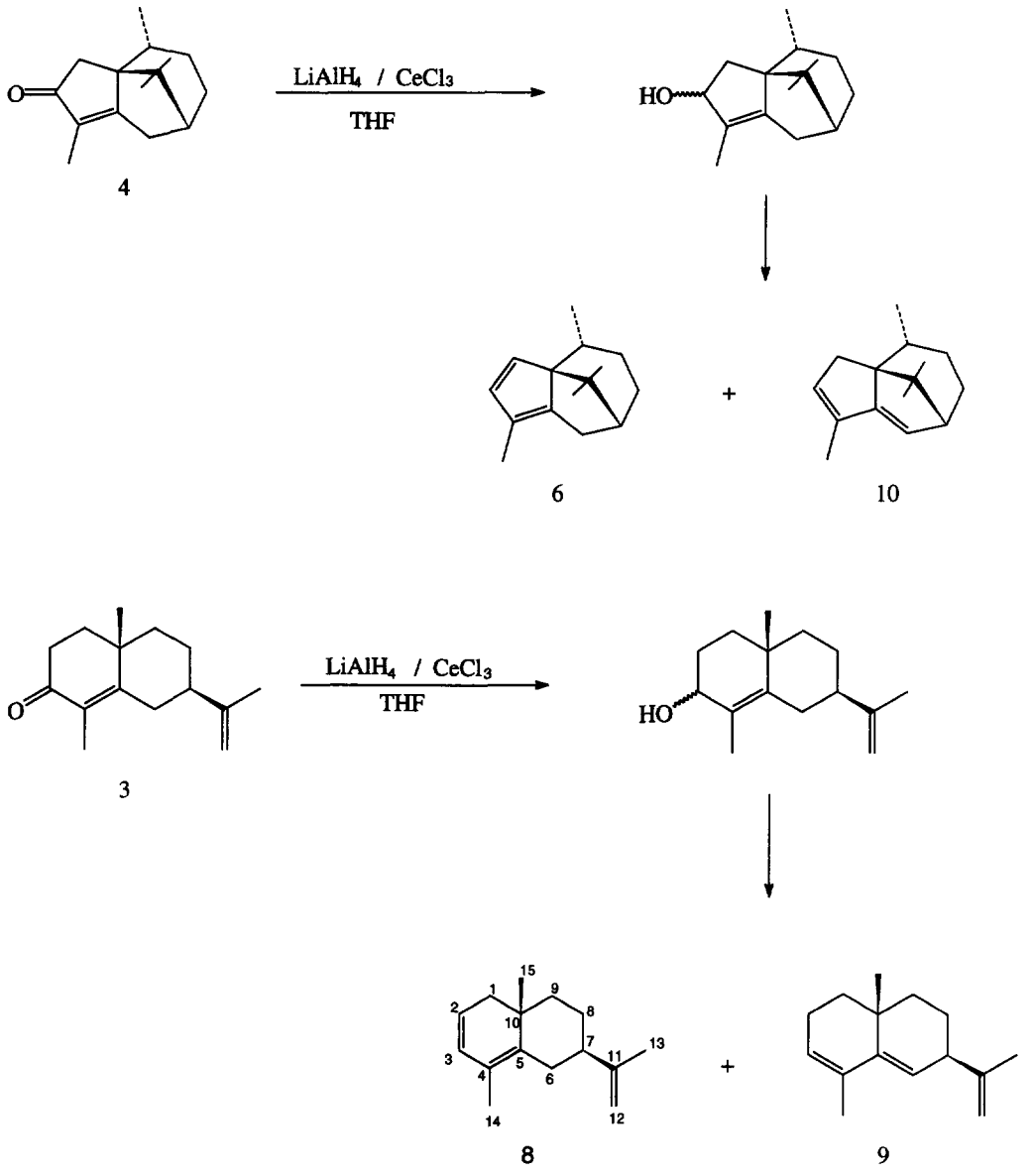

Fig. 2. Reduction of cyperotundone (**4**) and α-cyperone (**3**) and dehydration of the resulting alcohols to **6** and **10**, and **8** and **9**, respectively.

0.92 (1H, *s*), 1.38 (1H, *dd*, $J_1 = 12.71$ Hz, $J_2 = 3.56$ Hz), 1.48 (1H, *dd*, $J_1 = 12.71$ Hz, $J_2 = 3.56$ Hz), 1.65–1.70 (2H, *m*), 1.71 (3H, *s(br)*), 1.77 (3H, *s(br)*), 1.86 (1H, *d(br)*, $J = 14.50$ Hz), 1.93 (1H, *dt(br)*, $J_1 = 6.61$ Hz, $J_2 = 3.05$ Hz), 1.96 (1H, *dd*, $J_1 = 17$ Hz, $J_2 = 6.61$ Hz), 2.10 (1H, *d(br)*, $J = 17$ Hz), 2.60 (1H, *dt*, $J_1 = 14.50$ Hz, $J_2 = 2.55$ Hz), 4.72 (2H, *m*), 5.66 (1H, *ddd*, $J_1 = 8.5$–9 Hz, $J_2 = 6.1$ Hz, $J_3 = 2.03$ Hz), 5.72 (1H, *dd*, $J_1 = 8.5$–9 Hz, $J_2 = 3.05$ Hz); MS (EI, 70 eV), *m/z* (rel. int.): 202(23) $[M]^+$, 187(9), 173(1), 159(9), 145(32), 131(100), 119(43), 105(60), 91(31), 77(18), 67(9), 63(2), 55(14), 51(5), 41(29).

3,5,11-*Eudesmatriene* (**9**). ^{1}H NMR (400 MHz): δ 0.99 (3H, *s*), 1.36–1.48 (3H, *m*), 1.49–1.56 (1H, *m*), 1.60–1.76 (4H, *m*), 1.78 (3H, *m*), 2.05 (1H, *m*), 2.26 (1H, *m*), 2.91 (1H, *t*, $J = 8.14$ Hz), 4.73 (1H, *m*), 4.77 (1H, *m*), 5.41 (1H, *m*), 5.52 (1H, *m*); MS (EI, 70 eV), *m/z* (rel. int.): 202(100) $[M]^+$, 187(91), 173(15), 161(12), 145(26), 131(30), 121(41), 115(14), 105(39), 91(39), 77(24), 67(12), 63(4), 55(17), 51(8), 41(36).

Epoxycyperene (**7**). ^{1}H NMR (400 MHz): ^{1}H NMR: δ 0.82 (3H, *s*), 0.91 (3H, *s*), 1.05 (3H, *d*, $J = 7.12$ Hz), 1.25 (3H, *s*), 1.38–1.46 (2H, *m*), 1.55–1.70 (5H, *m*), 1.83–2.5 (5H, *m*); ^{13}C NMR 16.51, 18.66, 1962, 23.78, 25.25, 27.80, 28.22, 29.25, 32.99, 33.91, 42.82, 45.77, 55.58, 67.50, 82.20; MS (EI, 70 eV), *m/z* (rel. int.): 220(7) $[M]^+$, 205(47), 187(8), 177(18), 159(22), 147(23), 133(19), 119(100), 105(32), 91(28), 69(16), 65(8), 55(25), 51(5), 41(56).

Epoxidation of cyperene. To a stirred dispersion of cyperene (10 mg) in deionised H_2O powdered *m*-chloroperoxybenzoic acid (excess) was added at 0° and the

mixt. was stirred at room temp for 2 h. The soln was then neutralized with diluted NaOH and extracted with Et_2O.

Reduction of cyperotundone (**4**) *and* (+) *α-cyperone* (**3**). Reduction of the two α,β-unsaturated ketones was achieved with $LiAlH_4$ in the presence of $CeCl_3$ [12].

Dehydration of the obtained alcohols. Dehydration occurs in the injection port (200°) of the gas chromatograph, although a small quantity of alcohol was still present.

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REFERENCES

1. Uppal, S. K., Chhabra, B. R. and Kalai, P. S., *Phytochemistry*, 1984, **23**, 2367.
2. Couchman, F. M., Pinder, A. R. and Bromham, N. H., *Tetrahedron*, 1964, **20**, 2037.
3. Garbarino, J. A., Gambaro, V. and Chamy, M. C., *Journal of Natural Products*, 1985, **48**, 323.
4. Trivedi, B., Motl, O., Smolikova, J. and Sorm, F., *Tetrahedron Letters*, 1964, 1197.
5. Panikar, S. K., Motl, O. and Chakravarti, K. K., *Tetrahedron Letters*, 1977, **24**, 2121.
6. Howe, R. and McQuillin, F. J., *Journal of the Chemical Society*, 1955, 2423.
7. Hikino, H., Ito, K., Aota, K. and Takemoto, T., *Chemical and Pharmaceutical Bulletin*, 1966, **14**, 890.
8. Kapadia, V. H., Naik, V. G., Wadia, M. S. and Dev, S., *Tetrahedron Letters*, 1967, **47**, 4661.
9. Hardt, I. and König, W. A., *Journal of Chromatography*, 1994, **A 666**, 611.
10. Kubeczka, K.-H., Motl, O. and Formacek, V., *Proceedings of the 20th International Symposium on Essential Oils*. Würzburg, Germany, 1989.
11. Gopichand, Y., Pednekar, P. R. and Chakravarti, K. K., *Indian Journal of Chemistry*, 1978, **16B**, 148.
12. Fukuzawa, S., Fujinami, T., Yamauchi, S. and Sakai, S., *Journal of the Chemical Society, Perkin Transactions, I*, 1986, 1929.