Caparratriene, an Active Sesquiterpene Hydrocarbon from *Ocotea caparrapi*

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Received March 17, 1995[⊗]

Caparratriene (**1**), a new sesquiterpene hydrocarbon with significant growth inhibitory activity ($IC_{50} = 3.0 \pm 0.5 \times 10^{-6}$ M) against CEM leukemia cells, was isolated from the oil of *Ocotea caparrapi* (Nates) Dugand. The structure of **1**, determined by spectroscopic techniques, corresponded to (*E*,*E*)-3,7,11-trimethyl-2,4,10-dodecatriene ($C_{15}H_{26}$).

Ocotea caparrapi (Nates) Dugand (Lauraceae) is a large tree (ca. 25 m) native to the humid area surrounding the town of Caparrapi, Colombia. Its oil, extracted from a deep incision in the tree's trunk, has been used to treat insect and snake bites, skin ulcers, and other epithelial conditions including gonorrhea; it has also been used to treat bronchitis, laryngitis, and cancerous tumors. In most of these applications, a combination of external and internal treatment is commonly employed.[1] Partial chemical studies of the oil have appeared in the literature since 1898,[2] but no validation of components with therapeutic activity has been reported.

Previous studies[3] revealed nerolidol (**2**) as the main component of the oil (up to 70%), along with the bicyclic ether caparrapi oxide, assumed to be formed by cationic cyclization of **2**.[4] The oil also contained caparrapidiol and caparrapitriol, sesquiterpene alcohol homologs of **2**.[5] Chemical syntheses have confirmed the proposed structures of caparrapidiol[6] and caparrapi oxide.[7] Two more components, hydroxyotobain and reticuline, were reported in 1980.[8] None of the compounds found has any documented physiological activity. A renewed folk interest in Colombia for the use of this oil, especially in the treatment of cancerous conditions, has prompted us to re-examine this old remedy in search of verification of its anticancer activity and of its active component(s).

In a comparative study, Appel *et al.*[3] analyzed five samples of caparrapi oil of varied origin and found notable differences in their composition, with the most complex sample (sample 4) resembling the material reported here, which displayed approximately 17 components by GC-MS.

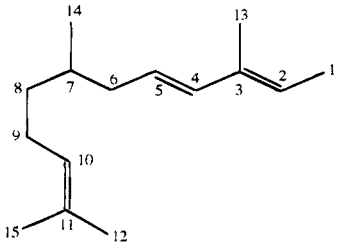
Cytotoxicity studies[9] of the oil using human leukemia cells (CEM) showed 23% inhibition of growth at 10^{-5}% concentration in a DMSO suspension. Although modest, this result was significant inasmuch as the oil, as well as its subsequent nonpolar fractions, displayed poor solubility in the aqueous growth medium. Fractionation of 100 g of the oil using a combination of hexanes, EtOAc, and EtOH as eluents produced four fractions that were subjected to the same cytotoxicity bioassay.

Fraction I (6%), obtained by extraction and CC on Si gel with hexanes as eluent, showed four compounds by TLC. Two of these, previously reported by not characterized,[3] were uv absorbers (254 nm) with apparent molecular ions of 206 and 220 by MS. Fractions II and III, eluted with a mixture of hexanes and EtOAc, contained **2** (ca. 70%) and caparrapi oxide. Fraction IV was eluted with a mixture of EtOAc and EtOH to give caparrapidiol and caparrapitriol. The identification of these compounds was made by comparing their R_f values and MS fragmentation patterns with literature reports.[3]

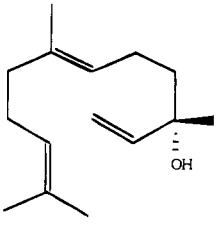
Fractions I – IV were tested for cytotoxicity. Fraction I displayed 85% growth inhibition at 10^{-5}% concentration, despite its poor solubility in the growth medium. Of the remaining fractions, only fraction IV was mildly cytotoxic (55% inhibition at 10^{-3}% concentration).

The main component of fraction I was isolated in 94% purity using KC18 (250 μ) reversed-phase TLC plates with a mixture of CH_3CN-H_2O (20:1.5) as the eluent. IR analysis of this material indicated a simple hydrocarbon with bands at 2910–3000, 1450, and 1372 cm^{-1}. DEPT[10] indicated two quaternary olefinic carbons, five tertiary carbons (four olefinic), three secondary carbons, and five methyl groups. HRMS of this sesquiterpene hydrocarbon afforded a molecular mass of 206.2030 (expected 206.2034). These results established a molecular formula of $C_{15}H_{26}$. In addition, the UV absorbing characteristics of this material (maximum absorbance 246 nm) indicated a conjugated system.

Further analyses using NMR (HOMODEC), HETCOR, INAPT, and selective NOE, provided the connectivity and isomerism that conforms to a hydrocarbon we have named caparratriene (**1**). Table 1 lists ^{1}H- and ^{13}C-NMR absorptions, as well as INAPT cross peaks. A detailed description of the connectivity assignments follows.



1 Caparratriene



2 Nerolidol

Five methyl groups were observed in the ^{1}H NMR, one of them (H-14) connected to a saturated tertiary

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1995.

0163-3864/96/3259-0077$12.00/0

Table 1. NMR (500 MHz) Data for Compound **1** in $CDCl_3$

proton	ppm (J = Hz)	INAPT[a] ^{13}C cross peaks	carbon	ppm
1	1.718 (d, 3H, J = 7.0)		1	13.633
2	5.45 (q, 1H, J = 7.0)	1, 13, 4	2	124.271
4	6.056 (d, 1H, J = 15.7)	13, 2, 3, 6	3	134.477
5	5.54 (dt, 1H, J = 15.5, 15.7)	3, 6, 7	4	135.858
6a	1.94 (m, 1H)		5	125.667
6b	2.10 (m, 1H)		6	40.338
7	1.53 (m, 1H)		7	33.101
8a	1.17 (m, 1H)		8	36.727
8b	1.37 (m, 1H)		9	25.641
9	1.99 (m, 2H)		10	124.921
10	5.12 (t, 1H, J = 7.0)	15, 8, 9, 11, 12	11	130.984
12	1.700 (s, 3H)		12	25.701
13	1.743 (s, 3H)		13	12.090
14	0.897 (d, 3H, J = 7.0)		14	19.467
15	1.621 (s, 3H)		15	17.592

[a] Obtained by positioning decoupler at the corresponding proton frequencies.

carbon as indicated by its proton chemical shift (0.897 ppm) and coupling (doublet, J = 7.0 Hz). The remaining four methyl groups were olefin-bound, three of them were connected to quaternary carbons as shown by DEPT and HETCOR spectra. The fourth methyl group was attached to a tertiary carbon, as indicated by its δ value (1.718 ppm) and coupling (doublet, J = 7.0 Hz), and therefore, suggested to be one of the termini of the molecule (C-1). The protons at C-1 were coupled with an olefinic proton at 5.45 ppm (H-2), because irradiation at that proton in a HOMODEC experiment altered the doublet at 1.718 ppm (H-1). The fact that H-2 was an unambiguous quartet, suggested that C-2 was connected to a quaternary olefinic carbon (C-3), which could be linked to a methyl group. To validate this assumption, an INAPT experiment was performed. This technique provides information on long-range H—C connectivity, by showing the transfer of proton magnetization to any ^{13}C nucleus that has significant long-range scalar interaction with an irradiated proton. Thus, irradiation of H-2 induced magnetization on a primary carbon at 12.090 ppm (assigned as C-13) confirming its connectivity to C-3. Induction of magnetization was also observed on C-1 and on a carbon at 135.858 ppm, assigned as C-4. This last carbon is tertiary olefinic (based on DEPT and δ value), whose proton, H-4, at 6.056 ppm was displayed as a doublet with a J value (15.75 Hz) indicative of a *trans*-olefinic coupling.

Irradiation of H-4 in the INAPT experiment, induced magnetization on C-13, C-2, and C-3 and on a methylenic carbon at 40.338 ppm, assigned as C-6. Thus, C-5 was assigned primarily by the coupling of its H-5 proton (J = 15.7 Hz) with H-4 and by irradiation of H-4 in a HOMODEC experiment that altered H-5 coupling. The connectivity between C-5 and C-6 was given by HOMODEC irradiation at H-5, which altered multiplets at 1.94 ppm and 2.10 ppm, indicating that protons at methylenic C-6 (from DEPT) were diastereotopic. This observation was confirmed by HETCOR, where the two proton signals are seen as sharing the same ^{13}C axis, an by NOE enhancements of 2% and 1.6% on these protons upon irradiation on H-5.

Irradiation of H-5 (INAPT) induced magnetizations on C-6, on C-3, and on a carbon at 33.101. This last carbon was assigned as the tertiary asymmetric C-7, which has a methyl group (C-14) attached to it. Irradiation of H-7 in HOMODEC mode confirmed this observation by altering the absorptions of H-14, H-6a, H-6b, and two more signals at 1.17 ppm and 1.37 ppm. These last proton signals shared the same ^{13}C axis in the HETCOR spectrum and were assigned as the diastereotopic protons H-8a and H-8b. Further irradiations of H-8a and H-8b in HOMODEC mode confirmed the connectivity between C-7 and C-8 and showed at the same time the coupling of these protons with protons at C-9. The latter, C-9, is attached to an olefinic tertiary C-10, as suggested by the δ value of its protons (H-9, 1.99 ppm) and by a HOMODEC experiment on H-10. The signal of H-9 in the HETCOR spectrum is shown as sharing the same ^{13}C axis with H-13 (1.743 ppm), but they were differentiated on the DEPT spectrum. The unambiguous triplet of H-10 suggested that C-10 was attached to a quaternary olefinic carbon, C-11. Irradiation of H-10 in an NOE experiment produced 2.5% enhancement on H-9, as well as enhancements on H-8a, H-8b, and an 8% enhancement on a methyl group at 1.700 ppm. This last absorption was assigned as H-12 on a methyl group that should then share C-11 with the methyl carbon C-15 (17.592 ppm), and that was assigned as another terminus of the molecule. In addition, the methyl carbon C-12 should be on cis relation with H-10 as suggested by its NOE enhancement. Confirmation of these last connectivities was obtained by an INAPT experiment on H-10, in which magnetization transfer was observed on C-15, C-8, and C-12/9 (differentiation of C-12 from C-9 is not observed here but in DEPT, therefore, induction on C-9 is not well defined).

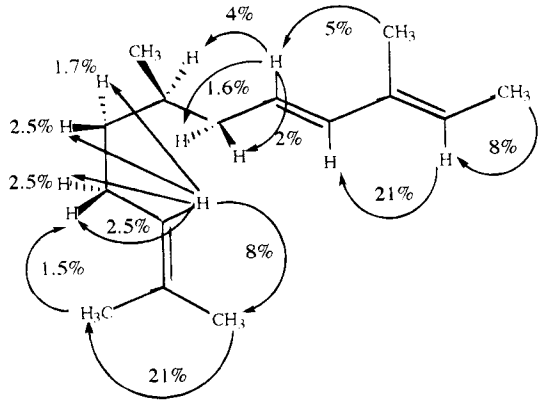


Figure 1. NOE enhancements for **1**.

A compilation of the enhancements observed by NOE are shown in Figure 1. Fragmentation patterns of **1** under electron impact conditions conformed with the assigned structure.

Caparratriene (**1**) is a colorless oil with a faint, pleasant, floral odor, stable at low temperature (0 °C). In vitro testing using human leukemia cells (CEM), **1** displayed growth inhibitory activity with an IC_{50} of 3.0 $\pm$ 0.5 $\times$ 10^{-6} M. Although modest, this inhibitory value is significant given the fact that **1** was poorly soluble in the testing medium and its evaluation had to be performed as a suspension in DMSO. An increase in inhibitory activity was observed when the treated cell cultures were exposed to light at short intervals during the incubation period. This observation, which may be linked to its mechanism of action, is currently under investigation. Natural and synthetic sesquiterpene hydrocarbons isomeric with **1** have been reported in the

literature,[11,12] but none contains, to our knowledge, a conjugated diene substructure.

Experimental Section

General Experimental Procedures. All NMR spectra (^1H and ^{13}C, HOMODEC, HETCOR, NOE, and INAPT) were obtained on a Varian 500 FT spectrometer. GC–MS spectra were taken on a Hewlett Packard HP 5890A gas chromatograph equipped with a 30-m DB5 column and connected to a HP 5970B mass spectrometer. Mass spectra were obtained in a gas reservoir with a Kratos MS 80 RFA spectrometer at 70 eV ionization potential and peak matching at a resolution of 10,000. UV spectra were recorded on a Perkin-Elmer lambda 5 UV/VIS spectrometer, and IR spectra were taken on a Perkin-Elmer 1330 spectrometer. Optical rotation was measured in hexane solution on a JASCO DIP-370 polarimeter. Analytical TLC was carried out on Aldrich Si gel F_{254} plastic sheets with hexanes or hexanes-ethyl acetate (1:1) as eluents. Reversed-phase prep. TLC was performed on Aldrich 250μ KC18 plates using CH_3CN-H_2O (20:1.5) as the eluent.

Source Material. The oil was purchased in the open market in the town of Caparrapi, Colombia. An assessment of its quality was performed by comparison with previously reported compositions,[3] as determined by GC–MS and TLC on Si gel.

Sample Fractionation and Isolation. Four fractions were obtained from 100 g of the oil by CC on Si gel. The solvent system, applied sequentially, consisted of: (1) hexanes, (2) hexanes-EtOAc (1:1), (3) hexanes-EtOAc (1:4), and (4) EtOAc-EtOH (4:1). Fraction I (6.0 g, 6% yield), obtained by elution with hexanes, showed four compounds by TLC and was further separated by reversed-phase TLC on KC18 (250 μ) silica plates using CH_3CN-H_2O (20:1.5) as the eluent. Compound **1** (4.5 g, 4.5% overall yield), the main component of this fraction had an R_f of 0.36 in this solvent system. The purity of **1** was assessed by ^1H 500 MHz NMR.

***Caparratriene* (1):** IR (neat) 2910–3000 (aliph. CH), 1450 (CH_2), and 1372 (CH_3) cm^{-1}; $\{\alpha\}^{25}_D$ +18.1 (c=0.1, CH_2Cl_2); UV λ max (hex) 246 with minor absorbances at 269 and 280; HRMS *m/z* $[M]^+$ 206.2030 ($C_{15}H_{26}$ requires 206.2034); EIMS *m/z* (rel. int.) $[M]^+$ 206(10), 191(9), 177(5), 163(20), 149(8), 136(22), 121(43), 109-(58), 95(75), 81(42), 69(100), 55(58); NMR data, see Table 1.

In Vitro Testing. Evaluation of cytotoxicity was performed using standard methodology[9] on human CEM cells grown on RPMI 1640 (Sigma) with 10% bovine calf serum and 1% penicillin/streptomycin (Sigma). Cells (300,000) in 1 mL of medium were placed in 24 × 2 mL wells and added with 20 μL of 5 testing solutions or suspensions of each fraction in DMSO at concentrations ranging from 10^{-1} to 10^{-5}% for mixtures, or 10^{-2} to 10^{-6} M for purified materials. After incubation for 24 h at 37 °C and 5% CO_2, cells were counted visually in a hemacytometer using trypan blue as indicator of survival, and the count compared with controls run under the same conditions. Materials with IC_{50} values equal to or better than 10^{-4}% were considered for further evaluation. Compounds with IC_{50} values of 10^{-5} M or better were considered active. Experiments for active materials were performed five times. Mechlorethamine and 5-FU were used as reference controls.

Acknowledgment. We thank Dr. Jatzmina Vivas for acquiring the oil used in this investigation.

References and Notes

(1) Garcia Barriga, H. *Flora Medicinal de Colombia*; Tercer Mundo: Bogota, 1992; Vol. 1, p 364.
(2) Tapia, M. *Bull. Soc. Chim. France* **1898**, *19*, 638.
(3) Appel, H. H.; Brooks, C. J. W., Campbell, M. M. *Perf. Essen. Oil Rep.* **1967**, 776.
(4) Brooks, C. J. W., Campbell, M. M. *Phytochemistry* **1969**, *8*, 215.
(5) Borges del Castillo, J., Brooks, C. J. W., Campbell, M. M. *Tetrahedron Lett.* **1966**, *31*, 3731.
(6) Weyerstahl, P.; Zummack, W. *Chem. Ber.* **1975**, *108*, 377.
(7) Cookson, R. C.; Lombardi, P. *Gazz. Chim. Ital.* **1975**, *105*, 621.
(8) Cuca Suarez, L. E. *Rev. Latinoam Quim.* **1980**, *11*, 110.
(9) Adams, R. L. P. *Cell Culture for Biochemists*, 2nd Ed.; Elsevier: New York, 1990, pp 63–80.
(10) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.
(11) Birch, A. J.; Chamberlain, K. B.; Moore, B. P.; Powell, V. H. *Aust. J. Chem.* **1970**, *23*, 2337.
(12) Nishino, C.; Bowers, W. S. *Tetrahedron* **1976**, *32*, 2875.

NP960012R