Cupaniol, a New Branched Polyprenol, from *Cupania latifolia*

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Received March 9, 2005; accepted April 18, 2005

A new branched polyprenol, designated cupaniol, has been isolated from the methanol extract of the leaves of *Cupania latifolia* (Sapindaceae). The structure was determined to be (2*E*,6*E*,12*E*,16*E*)-3,7,13,17,21-pentamethyl-10-(1-methylethenyl)-2,6,12,16,20-docosapentaen-1-ol on the basis of spectral analysis and conversion to a known compound.

Key words cupaniol; *Cupania latifolia*; Sapindaceae; branched polyprenol

Cupania latifolia KUNTH [syn. *C. americana* subsp. *latifolia* (KUNTH) T. D. PENN., *C. papillosa* RADLK., and *C. sedimentata* RADLK.] of the family of Sapindaceae is a medium-sized tree that grows in rainforests of Caribbean countries to Peru in South America. The plant is called *guara*, *mestizo*, or *guacharaco* in Colombia and used to give shade on coffee plantations and for ornamental purposes. The leaves of the related species *C. americana*, which also grows in Mesoamerican rainforests, have been used as a painkiller and the seeds for the treatment of dysentery.[1)] Only a few phytochemical studies on the genera *Cupania* have been reported.[2)] To our knowledge, neither *C. americana* nor *C. latifolia* has been chemically investigated. In this paper, we report the isolation and structure elucidation of a new branched polyprenol (**1**) (Fig. 1), designated cupaniol, from the leaves of *C. Latifolia*, collected in Manizales, Caldas, Colombia.

Results and Discussion

Chromatographic separation, including silica gel and Sephadex LH-20 columns, of the ethyl acetate (AcOEt)-soluble part of the methanol (MeOH) extract yielded compound **1** as a colorless oil. The molecular formula of **1** was determined to be $C_{30}H_{50}O$ on the basis of high-resolution (HR)-EI-MS data (observed: 426.3853; $C_{30}H_{50}O$ requires 426.3862). The ^{1}H-NMR spectrum showed signals of five olefinic protons, exomethylene protons, oxymethylene protons, and seven singlet methyl groups on double bonds, among others. The signal of the oxymethylene protons (δ 4.15) was coupled to an olefinic proton, as revealed by the H–H correlation spectroscopy (COSY) spectrum. The ^{13}C-NMR spectrum displayed 30 peaks that were classified, assisted by distortionless enhancement by polarization transfer (DEPT) experiments, into 12 olefinic carbons, one oxymethylene carbon (δ 59.37), seven methyl, one aliphatic methine (δ 47.12), and nine aliphatic methylene carbons. The presence of the CH carbon was noteworthy. The heteronuclear multiple-bond correlation (HMBC) spectrum allowed connection of the methine carbon (C-12) to an isopropenyl group (C-13, -14, -15) and an ethylene group (C-11, -10) (Fig. 2). The connection to the ethylene group was supported by the H–H COSY spectrum, which showed cross-peaks between H-12 and H_2-11 (δ_H 1.38, 1.46, δ_C 31.01), and between H_2-11 and H_2-10 (δ_H 1.9, δ_C 37.40). The C-10 was

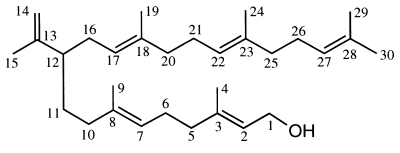

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Fig. 1. Chemical Structure of Cupaniol

Table 1. ^{1}H- and ^{13}C-NMR Data for **1** (500/125 MHz, $CDCl_3$)

C no.	^{1}H	^{13}C	HMBC correlation from H to C
1	4.15 (d, J=6.9 Hz)	59.37 (CH_2)	C-2, C-3
2	5.41 (t, J=6.9 Hz)	123.26 (CH)	C-1, C-4, C-5
3	—	139.80 (C)	
4	1.68 (s)	16.17 (CH_3)	C-2, C-3, C-5
5	1.94—2.12 (m)	39.54 (CH_2)	C-2, C-3, C-4
6	1.94—2.12 (m)	26.25 (CH_2)[a)]	*f)*
7	5.07—5.12 (m)	123.54 (CH)[b)]	C-10[f)]
8	—	135.62 (C)	
9	1.58 (s)	15.97 (CH_3)	C-7, C-8, C-10
10	1.9 (m)	37.40 (CH_2)	C-7, C-8, C-9, C-11, C-12
11	1.38/1.46 (m)	31.01 (CH_2)	C-10, C-12, C-13
12	1.94—2.12 (m)	47.12 (CH)	C-13, C-14, C-15
13	—	147.66 (C)	
14	4.65 (s), 4.74 (s)	111.23 (CH_2)	C-12, C-13, C-15
15	1.61 (s)	18.55 (CH_3)	C-12, C-13, C-14
16	1.94—2.12 (m)	32.14 (CH_2)	C-11, C-12, C-13
17	5.07—5.12 (m)	123.04 (CH)[b)]	C-12, C-16
18	—	135.35 (C)[c)]	
19	1.59 (s)	15.97 (CH_3)[d)]	*f)*
20	1.94—2.12 (m)	39.77 (CH_2)[e)]	*f)*
21	1.94—2.12 (m)	26.63 (CH_2)[a)]	*f)*
22	5.07—5.12 (m)	124.21 (CH)	*f)*
23	—	134.84 (C)[c)]	
24	1.59 (s)	16.25 (CH_3)[d)]	*f)*
25	1.94—2.12 (m)	39.70 (CH_2)[e)]	*f)*
26	1.94—2.12 (m)	26.74 (CH_2)[a)]	*f)*
27	5.07—5.12 (m)	124.37 (CH)	C-29, C-30
28	—	131.22 (C)	
29	1.605	17.67 (CH_3)	C-27, C-28, C-30
30	1.68 (s)	25.69 (CH_3)	C-27, C-28, C-29

a—e) Interchangeable. *f*) Expected correlations were observed, but it was difficult to assign them due to overlapping of proton and/or carbon signals.

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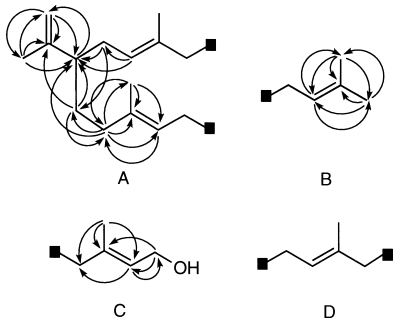


Fig. 2. Partial Structures for Cupaniol as Revealed by 2D-NMR Studies
Arrows represent HMBC correlations (H→C).

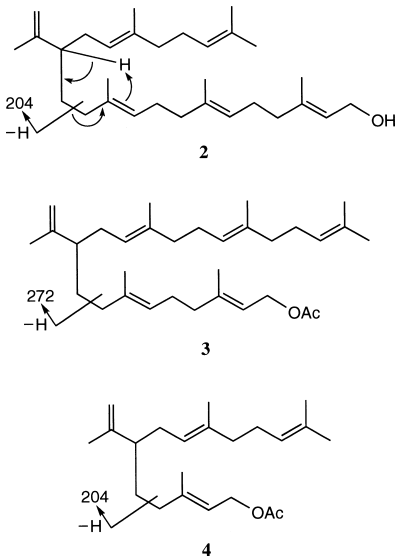
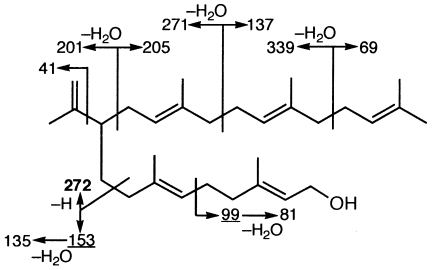


Fig. 3. Reported Intense Fragment Ions for **3** and **4**, and an Expected Fragment Ion for **2**



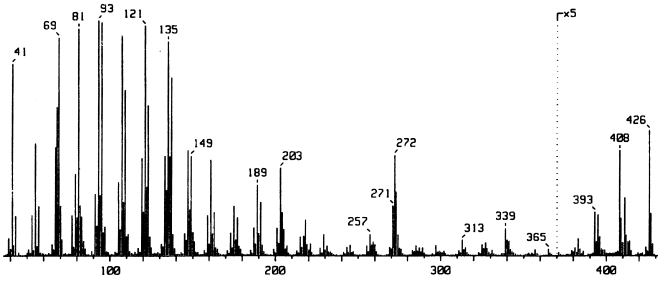


Fig. 4. EI-MS (70 eV) Spectrum and Fragmentation Pattern of Compound **1**
The underlined fragment ions could not be observed.

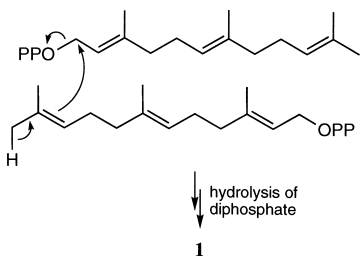


Fig. 5. Postulated Biosynthesis of Compound **1**
OPP represents diphosphate group.

suggested to be a carbon of a prenyl unit (C-6 to C-10), since a methyl group (δ_H 1.58, H_3-9) was correlated with the C-10 carbon and two olefinic carbons at 135.62 (C-8) and 123.54 (C-7). In addition, H-12 was further correlated with C-16 (δ_C 32.14) and C-17 (δ_C 123.04) in the HMBC and H–H COSY spectra. Another prenyl unit involving C-16 and C-17 could be incorporated into the partial structure **A**, since HMBC correlations from a methyl group (H_3-19) to C-17, C-18, and C-20 were observed. The presence of the two terminal isoprene units **B** and **C** was readily deduced from the HMBC correlations depicted in Fig. 2. The three partial structures **A**—**C** accounted for 25 carbons of the molecule, thus suggesting the presence of yet another prenyl unit **D**. A linear combination of the four partial structures, keeping in mind the biosynthetic isoprene rule, would give rise to the two possible structures **1** and **2** (Fig. 3) for cupaniol.

Although analysis of the NMR data did not afford further information on the structure, the EI-MS spectrum provide a clue as to whether **1** or **2** is the correct structure of cupaniol. Fortunately, EI-MS fragmentation patterns of synthetic related compounds **3** and **4** were reported previously: An intense fragment ion due to McLafferty rearrangement was observed at *m*/*z* 272 and 204 for compounds **3** and **4**, respectively.[3] It is therefore expected that structure **1** should afford an intense fragment ion at *m*/*z* 272 (see Fig. 4), while structure **2** should yield an ion at *m*/*z* 204 (Fig. 3). The EI-MS of cupaniol is illustrated in Fig. 4, which clearly shows a fragment ion at *m*/*z* 272 (HR-EI-MS *m*/*z*: 272.2520; $C_{20}H_{32}$ requires 272.2502), consistent with the structure **1**. The presense of the *m*/*z* 272 ion in the trimethylsilyl ether derivative of **1** further supported the interpretation of the ion.[4] The mass fragmentation of **1** is summarized in Fig. 4. The geometry of the double bond, *i.e.*, Δ^2, Δ^7, Δ^{17}, and Δ^{22} was determined to be all *trans* by comparing the ^{13}C data with those of relevant polyprenols.[5] Hence the structure of cupaniol was determined to be **1** as shown in Fig. 1.[6] Cupaniol had $[\alpha]_D$ +2.5° (c=2.1, $CHCl_3$), suggesting that this natural product is optically active, although the absolute configuration was not investigated. Finally, compound **1** was converted to the corresponding acetate, which was reported as a synthetic material.[3] The NMR and MS data of cupaniol acetate were in excellent agreement with the reported values.

Cupaniol **1** is a branched polyprenol. The simplest natural compound of this class is lavandulol, a dimer of isopentenol which occurs frequently in essential oils,[7,8] and cupaniol can be regarded as a higher homologue of lavandulol. There are a few examples of such higher homologues that were subjected to further modifications.[9,10] Hydrocarbon forms (arising from elimination of water and/or saturation and unsaturation) of related branched isoprenoids have been also reported in

coastal and deep-sea marine sediments[11,12] and benthic diatoms.[13] Biogenesis of **1** can be outlined as shown in Fig. 5.

In addition to cupaniol, ficaprenol 12,[14,15] α- and β-tocopherols,[16] β-amyrin,[17] and taraxerol[17] were isolated from the AcOEt-soluble fraction and characterized by comparing the NMR and MS data with the reported values, while (−)-epicatechin[18,19] and proanthocyanidin A2[20] were isolated from the AcOEt-insoluble fraction of the MeOH extract.

Experimental

General ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker DRX500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in $CDCl_3$ solutions with tetramethylsilane (TMS) as an internal reference. Chemical shifts are expressed in δ (ppm), referring to TMS (δ_H 0.00) and the solvent signal (δ_C 77.00). EI-MS (70 eV) spectra were obtained on a JEOL JMS-700 spectrometer in a direct inlet method. Optical rotations were measured on a JASCO DIP-360 polarimeter. Silica gel chromatography was carried out using Kieselgel 60 (E. Merck). HPLC was performed on a Shimadzu LC-6A instrument with an SPD-6A UV detector, using an ODS column (Shim-Pack CLC-ODS, 15 cm×6 mm i.d.).

Plant Material The leaves of *C. americana* were collected in October 2002 at Manizales, Caldas, Colombia. The plant was identified by Dr. Julio Betancur and a specimen voucher (No. COL495137) was deposited at the Instituto de Ciencias Naturales de la Universidad Nacional de Colombia.

Extraction and Isolation The air-dried leaves (1.0 kg) were extracted with methanol two times under reflux for 2 h. The concentrated MeOH (151 g) was shaken with AcOEt (400 ml×2) to give the AcOEt-soluble fraction (45 g) after removal of the solvent. This was chromatographed on silica gel with hexane–AcOEt. The fraction eluted with hexane–AcOEt (6 : 1) was chromatographed again under similar conditions. The resulting residue was separated on Sephadex LH-20 with AcOEt–$CHCl_3$ (1 : 1) as an eluent. The fraction enriched with compound **1** was finally separated by preparative TLC with hexane–AcOEt (7 : 1) as a developing solvent to yield **1** as a colorless oil (31 mg, 0.031% yield based on the air-dried leaves). Spectral data for **1** are described in the text.

The fractions eluted with hexane–AcOEt (10 : 1—8 : 1) in the above first chromatography were further chromatographed over silica gel and then separated on Sephadex LH-20 with hexane–AcOEt (1 : 1) to give ficaprenol 12 (150 mg). The fractions eluted with hexane–AcOEt (8 : 1—7 : 1) were further chromatographed on Sephadex LH-20 with AcOEt–$CHCl_3$ (1 : 1) and then separated by preparative TLC with hexane–ether (10 : 1) as a developing solvent to give partially purified α-tocopherol (7.5 mg) and β-tocopherol fractions. Final purification of the fractions by HPLC with methanol as an eluting solvent gave α-tocopherol (7.5 mg) and β-tocopherol (84 mg). The fractions eluted with hexane–AcOEt (7 : 1—6 : 1) were further chromatographed over silica gel and then separated on Sephadex LH-20 with $CHCl_3$–MeOH (2 : 3) to give a mixture of β-amyrin and taraxerol. The two triterpenes were finally separated by HPLC with methanol–THF (10 : 1) as an eluting solvent to yield β-amyrin (50 mg) and taraxerol (45 mg). The AcOEt-insoluble fraction of the original extract was chromatographed over silica gel with $CHCl_3$–MeOH. The fractions eluted with $CHCl_3$–MeOH (4 : 1) were further separated on Sephadex LH-20 with $CHCl_3$–MeOH (2 : 3) to give (−)-epicatechin (60 mg). The fractions eluted with $CHCl_3$–MeOH (3 : 1) were further separated on Sephadex LH-20 with $CHCl_3$–MeOH (2 : 3) to give proanthocyanidin A2 (405 mg).

Acknowledgments This study was supported by a grant-in-aid from the Ministry of Education, Science, Sports, Culture and Technology of Japan (No. 12576030), and from the Ministry of Environment, Housing and Territorial Development of Colombia and the National Coffee Growers of Colombia (Contract No. 21/2002). Thanks are also due to Dr. Julio Betancur for the identification of the plant.

References and Notes

1) Francis J. K., "*Cupania americana* L. Guara. SO-ITF-SM-544," US Department of Agriculture, Forest Service, Southern Forest Experiment Station, New Orleans, 1991, pp. 1—4.
2) Cavalcanti S. B. T., Teles H. L., Silva D. H. S., Furlan M., Young M., Claudia M., Bolzani V. S., *J. Brazilian Chem. Soc.*, **12**, 413—416 (2001).
3) Nagano H., Nakanishi E., Takajo S., Sakuma M., Kudo K., *Tetrahedron*, **55**, 2591—2608 (1999).
4) EI-MS of the trimethylsilyl ether derivative of **1**: *m*/*z* 498 (M^+, 5%), 483 (2), 429 (1), 408 (5), 272 (11), 271 (13), 255 (10), 203 (14), 156 (20), 135 (24), 121 (28), 81 (57), 69 (100), 55 (20), 41 (23).
5) "Carbon-13 NMR Spectroscopy," Chap. 5, ed. by Breitmaier E., Voelter W., VCH Verlagssgesellschaft, Weinheim, 1987.
6) IUPAC name: (2*E*,6*E*,12*E*,16*E*)-3,7,13,17,21-pentamethyl-10-(1-methylethenyl)-2,6,12,16,20-docosapentaen-1-ol.
7) Schinz H., Seidel C. F., *Helv. Chim. Acta*, **25**, 1572—1591 (1942).
8) Barazandeh M. M., *J. Essential Oil Res.*, **14**, 103—104 (2002).
9) Mangoni L., Merola D., Monaco P., Parrilli M., Previtera L., *Tetrahedron Lett.*, **25**, 2597—2600 (1984).
10) Bousserouel H., Litaudon M., Morleo B., Martin, Thoison O., Nosjean O., Boutin J. A., Renard P., Sevenet T., *Tetrahedron*, **61**, 845—851 (2005).
11) Robson J. N., Rowland S. J., *Nature* (London), **324**, 561—563 (1986).
12) Belt S. T., Cooke D. A., Hird S. J., Rowland S., *J. Chem. Soc., Chem. Commun.*, **1994**, 2077—2078 (1994).
13) *e.g.*, Belt S. T., Masse G., Allard W. G., Robert J.-M., Rowland S. J., *Organic Geochemistry*, **32**, 1271—1275 (2001).
14) Suga T., Ohta S., Nakai A., Munesada K., *J. Org. Chem.*, **54**, 3390—3393 (1989).
15) Aoki T., Matsuo K., Suga T., Ohta S., *Phytochemistry*, **46**, 715—720 (1997).
16) Strohschein S., Pursch M., Lubda D., Albert K., *Anal. Chem.*, **70**, 13—18 (1998).
17) Takasaki M., Konoshima T., Tokuda H., Masuda K., Arai Y., Shiojima K., Ageta H., *Biol. Pharm. Bull.*, **22**, 606—610 (1999).
18) Shen C.-C., Chang Y.-S., Ho L.-K., *Phytochemistry*, **34**, 843—845 (1993).
19) Foo L.-Y., Karchesy J. J., *Phytochemistry*, **28**, 1743—1747 (1989).
20) Lou H., Yamazaki Y., Sasaki T., Uchida M., Tanaka H., Oka S., *Phytochemistry*, **51**, 297—308 (1999).