Chemistry of Natural Compounds, Vol. 46, No. 4, 2010

MAIN CONSTITUENTS AND CYTOTOXIC ACTIVITY OF THE ESSENTIAL OIL OF *Piper artanthe*

E. Avella and J. Rios-Motta*

UDC 547.913

The identification of novel antitumor agents that demonstrate efficacy is necessary to improve medical treatment based on new drugs. The pharmaceutical industries have been working together in this area for several years to try to find novel compounds with good antitumor activity. Brine shrimp (*Artemia salina*) larvae have been reported as an organism that can detect bioactive compounds. Since there is a good correlation between the toxicity on *Artemia salina* and antitumor [1, 2], phototoxicity [3], and anti-*Trypanosoma cruzi* [4] activity, this test is a good prescreening method for identifying new potential antitumor agents.

Piper artanthe C.DC. (Piperaceae) is commonly known as the "cordoncillo." It is a shrub or small tree growing to 1 to 2 m, and it is widespread in America [5] and used in folk medicine for the treatment of different disease symptoms. Despite phytochemical studies on the *Piper* species describing the isolation of several bioactive compounds [6], to our knowledge *P. artanthe* has not been investigated for its biological activity, and no data can be found concerning the chemical composition of this species.

The main purpose of this study was not only to evaluate the biological activities using the *Artemia salina* toxicity model as a prescreening procedure but also to evaluate the composition and structural characterization of the main components of the essential oil from the aerial parts of *P. artanthe* C.DC.

As shown in Table 1, 41 compounds consisting of more than 87% of the total hydrodistilled essential oil were identified. The chromatographic data on both columns, HP-5MS and DB-1, indicate that the major components were apiol (14.52%), δ-elemene (11.69%), (*E*)-caryophyllene (10.24%), *epi*-cubebol (8.85%), myristicin (6.38%), and cubebol (6.30%). Over 44% of these compounds were sesquiterpenes, and 45% of them were oxygenated sesquiterpenes.

The evaluation of biological activity using the brine shrimp lethality test showed that the essential oil of the fresh aerial parts of *P. artanthe* displayed an LC_{50} value of 107.1 μg/mL (σ_{n-1}: 8.9 μg/mL). According to [7], crude extracts resulting in LC_{50} values of less than 250 μg/mL are considered significantly active. For that reason, this oil can be regarded as a promising candidate for further investigation. Accordingly, our following objective was to isolate and identify the major components of this essential oil in order to evaluate their individual biological activities. Though all constituents could not be isolated, the main component was made pure by means of column chromatography on silica gel as a yellowish oil (980 mg). This compound displayed an LC_{50} value of 13.1 μg/mL (σ_{n-1}: 0.4 μg/mL). Plant polyalkoxybenzenes display a broad spectrum of biological activities. For example, apiol has previously been reported to be a specific inhibitor of the biosynthesis of aflatoxin G1, a calcium antagonist, and a diuretic, abortive, sedative, antioxidant, and insecticidal agent [8–11].

^{1}H NMR spectrum analysis indicated that the main component isolated from *P. artanthe* essential oil was identical to apiol. However, a revision of the cited chemical shifts for the quaternary carbons (Table 2) [8, 9] showed that the complete assignment of its ^{13}C NMR spectrum was still ambiguous. In order to establish unambiguous ^{13}C NMR data for apiol, we re-acquired the ^{13}C NMR spectrum along with 2D NMR experimental evidence to resolve it. In the INADEQUATE spectrum of **1** the signal of C-1 (125 ppm) correlates with C-7 (33.6 ppm). The HMBC correlations from the benzylic CH_2 (2.54 ppm) to C-1 (125 ppm) and C-6 (108 ppm) further corroborated this assignment. Thus, our data are in accordance with the data of [9].

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker Avance 400 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) with TMS as an internal standard. Standard pulse sequences in Bruker XWINNMR (3.50 version) software were used for INADEQUATE (Jcc was set to be 50 Hz), 1D, and 2D experiments on the same instrument. Column chromatography (CC) was carried out on silica gel 60 F254 and silica gel 100 (Merck, Darmstadt, Germany).

Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Quimica, Santafe de Bogota, A. A. 14490, fax: +(571) 316 52 20, e-mail: jariosmo@unal.edu.co. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 547–549, July–August, 2010. Original article submitted March 25, 2009.

0009-3130/10/4604-0651

TABLE 1. Chemical Composition of the Essential Oil of Fresh Aerial Parts of *P. artanthe* Determined by GC/FID and GC/MS

Compound	%	RT[1]	KI[2]	Compound	%	RT[1]	KI[2]
Apiol*	14.52	70.63	1644	Sylvestrene**	0.50	24.74	1019
δ-Elemene**	11.69	48.38	1334	Tricyclene**	0.41	17.66	928
(*E*)-Caryophyllene*	10.24	54.16	1416	β-Funebrene**	0.41	54.43	–
epi-Cubebol**	8.85	59.16	1485	Cyclosativene*	0.40	50.07	1366
Myristicin mixed with cubebol**	6.38	60.72	1505	Caryophyllene oxide**	0.39	64.33	–
Cubebol**	6.30	60.46	1505	α-Guaiene**	0.29	56.78	1436
γ-Cadinene**	3.06	59.73	1493	Sabinene**	0.27	22.83	994
trans-β-Guaiene	2.90	59.36	1489	Spathulenol**	0.25	63.95	–
Myristicin mixed with δ-cadinene**	2.64	60.82	1508	*p*-Cymene*	0.24	24.38	1010
δ-Cadinene*	2.37	60.92	1513	Δ^2-Carene*	0.23	23,76	1007
γ-Muurolene**	2.27	58.01	1474	Germacren-4-ol**	0.21	63.78	–
α-Copaene*	2.25	50.85	1386	β-Selinene*	0.18	58.26	1481
cis-4(14),5-Muuroladiene**	2.05	57.36	1456	Hinesol**	0.18	66.76	
β-Cubebene**	1.15	51.75	1386	α-Muurolol**	0.15	67.93	
Aromadendrene*	1.15	56.62	1434	α-Terpinolene*	0.17	29.31	
Artemisiatriene*	1.03	20.84	967	Germacrene B**	0.12	62.71	
α-Cubebene*	0.85	48.94	1347	Isoledene**	0.12	50.17	1374
α-Santalene**	0.80	56.11	1425	Elemicin**	0.09	62.21	
γ-Terpinene*	0.60	27.08	1048	α-Calacorene**	0.08	61.82	
1,4-Cadinadiene**	0.59	61.30	1534	Linalool**	0.06	30.15	
Cubenol**	0.57	67.74	–				

RT: retention time (min) on HP-5MS capillary column. KI: kovatz indices on DB-1 capillary column.
*Analysis and comparison with mass spectra in the Wiley Registry of Mass Spectral Data [13]. **Analysis and comparison with mass spectra of authentic samples [14, 15].

TABLE 2. ^{13}C NMR Spectroscopic Data Reported for Apiol (**1**) [8, 9]

C atom	Ref. [8]	Ref. [9]	This work
1	**110.8**	**125.8**	**125.6**
2	136.5	136.2	136.3
3	139.0	138.7	138.7
4	135.2	135.1	135.2
5	139.2	139.1	139.1
6	108.5	108.1	108.5
7	34.3	34.1	34.1
8	137.6	137.3	137.4
9	115.6	115.4	115.2
2-OMe	60.4	60.1	59.8
5-OMe	57.1	55.8	56.7
OCH_2O	101.7	101.5	101.5

Precoated plates of silica gel 60 F254 (Merck, Darmstadt, Germany) were used for analytical purposes, and the spots were detected with a UV lamp at 254 and 366 nm and by spraying with 50% H_2SO_4 followed by heating.

Plant Material. *Piper artanthe* C.DC. was collected in San Miguel (Santander, Colombia). The plant specimen was identified and authenticated by Dr. Gustavo Lozano (Instituto de Ciencias Naturales, Universidad Nacional de Colombia). A voucher specimen (COL. 347949) was deposited at Herbario Nacional Colombiano.

Extraction and Isolation. Fresh aerial parts (leaves and stems) of the plant (641 g) were cut and submitted to hydrodistillation for 4 h using a Clevenger-type apparatus. The oil obtained was dried over anhydrous sodium sulfate. This oil (0.76% on a wet basis, ρ_{20}: 0.951 g/mL, η_D: 1.5052 at 20°C) was kept cold (0–4°C) in an amber glass flask for CC, FID-GC, and GC/MS analysis.

Four grams of essential oil was subjected to silica gel column chromatography (50 × 2.5 cm, 70–230 mesh, 200 g) and eluted with petroleum ether (40–60)–EtOAc (96:4). Fractions of 50 mL each were collected and combined on the basis of their TLC profiles to obtain either mixtures or an isolated pure component, apiol, eluating in the fractions numbered from 18 to 25 (980 mg, R_f 0.5, hexane–EtOAc 95:5, R_f 0.35, petroleum ether (40–60)–$CHCl_3$ 7:3).

Apiol (1), pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.24 (2H, d, J = 6.5, CH_2-7), 3.77 (3H, s, 5-OMe), 3.82 (3H, s, 2-OMe), 4.98 and 4.99 (2H, m, CH_2-9), 5.84 (2H, s, OCH_2O), 5.87 (1H, ddt, J = 16.7, 10.3, 6.5, H-8), 6.25 (1H, s, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): see data in Table 2.

Identification of Components. The compound identities were confirmed by comparing their KI and mass spectral data with the literature data and with those of the Wiley Registry of Mass Spectral Data [12–15].

Toxicity to *Artemia salina*. The protocol established by McLaughlin [1] was employed. Brine shrimp eggs (*Artemia salina*, Sanders Great Salt Lake Brine Shrimp Company L.C., USA) were hatched in artificial seawater prepared from commercial sea salt, 38 g/L. Stock solutions prepared with 2.0 mg of essential oil or of apiol (**1**) dissolved in 2.0 mL of dichloromethane were used. From these solutions, 1.0 mL, 100, and 20 μL of essential oil stock solution, or 500.0, 50.0, and 5.0 μL of apiol, were transferred to vials in triplicate. The solvent was removed at room temperature and seawater (5 mL) was added to each vial, resulting in final concentrations of 200, 20, and 4 μg/mL of essential oil, or 100.0, 10.0, and 1.0 μg/mL of apiol. Second instar larvae (48 h) of *A. salina* (ten per vial) were added. After 24 h of contact, the survivors were counted, and LC_{50} values with 95% confidence intervals were calculated by the Finney program [16].

Supporting Information. Copies of the original data and spectra that verify the content of this paper are obtainable form the corresponding author.

ACKNOWLEDGMENT

The authors are grateful to Dr. Elena Stashenko, Universidad Industrial de Santander, for CG-FID and CGEM, to the CYTED Program IV.6 for instructions about essential oil analysis, and to COLCIENCIAS (1101-05-028-97) and CINDEC-Universidad Nacional de Colombia (703525) for financial support.

REFERENCES

1. J. M. McLaughlin, *Crown Gall Tumours on Potato Discs and Brine Shrimp Lethality: Two Simple Bioassays for Higher Plant Screening and Fractionation*. In K Hostettmann, Assays for Bioactivity. Academic Press; San Diego, 1991, p. 2–32.
2. S. De Rosa, A. De Giulio, and C. Iodice, *J. Nat. Prod*., **57**, 1711 (1994).
3. T. Ojala, P. Vuorela, J. Kiviranta, H. Vuorela, and R. Hiltunen, *Planta Med*., **65**, 715 (1999).
4. C. L. Zani, P. P. G. Chaves, R. Queiroz, N. M. Mendes, A. B. Oliveira, J. E. Cardoso, A. M. G. Anjos, and T. S. Grandi, *Phytomedicine*, **2**, 47 (1995).
5. W. Trelease, *Proc. Am. Philos. Soc*., **69**, 309 (1930).
6. V. S. Parmar, S. C. Jain, K. S. Bisht, R. Jain, P. Taneja, A. Jha, O. D. Tyagi, A. K. Prasad, J. Wengel, C. E. Olsen, and P. M. Boll, *Phytochemistry*, **46**, 597 (1997).
7. M. J. Rieser, Z.-M. Gu, X.-P. Fang, L. Zeng, K. V. Wood, and J. L. McLaughlin, *J. Nat. Prod*., **59**, 100 (1996).
8. B. V. De O. Santos, E. V. L. Da-Cunha, M. C. De O. Chaves, and A. I. Gray, *Phytochemistry*, **49**, 1381 (1998).
9. M. Razzaghi-Abyaneh, T. Yoshinari, M. Shams-Ghahfarokhi, M. B. Rezaee, H. Nagasawa, and S. Sakuda, *Biosci. Biotech. Biochem*., **71**, 2329 (2007).
10. M. N. Semenova, D. V. Tsyganov, A. P. Yakubov, A. S. Kiselyov, and V. V. Semenov, *ACS Chem. Biol*., **3**, 95 (2008).
11. H. Zhang, F. Chen, X. Wang, and H.-Y. Yao, *Food Res. Int.*, **39**, 833 (2006).
12. P. Sandra and C. Bicchi, (eds), *Capillary Gas Chromatography in Essential Oil Analysis*, Huethig Buch Verlag, Heidelberg, 1987, p. 261.
13. F. W. McLafferty and D. B. Stauffer, *Registry of Mass Spectral Data, 6th Electronic Edition*, Wiley, New York, 1994.
14. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography-Mass Spectroscopy*, Allured Publishing Corporation; Illinois, 1995, p. 19.
15. B. M. Lawrence, B. D. Mookherjee, and B. J. Willis, *Flavors and Fragrances: a World Perspective. Proceedings of the 10th International Congress of Essential Oil, Fragances and Flavors*, Elsevier, Washington, 1986, p. 951.
16. D. J. Finney, *Probit Analisis*, 3rd edition., Cambridge University Press, Cambridge, 1971, p. 72.