Identification of Two Chromenes from *Calea serrata* by Semiautomatic Structure Elucidation

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The isolation and semiautomatic structure identification of the two chromenes, eupatoriochromene (**1**) and preconene II (**2**), from the aerial parts of *Calea serrata* is described. The structure elucidation was performed on the basis of 1D and 2D NMR methods using the recently published computer program LUCY. Neither compound has been isolated from *Calea serrata* previously.

Calea serrata Less. (Compositae) is endemic in the south of Brazil, where it is called "erva de cobra" ("snakeherb") or "quebra tudo" ("breaks anything"). The plant, which has a very bitter taste, is used in a religious ritual called "Umbanda" and has also various medical applications, for example, as a tea to cure stomach disorders such as ulcers and as a liver therapeutic agent.[1]

We now report the isolation and semiautomatic identification of two chromenes, eupatoriochromene (**1**) and preconene II (**2**), from *C. serrata.* The structure elucidation was performed on the basis of 1D- and 2D NMR experiments (broad-band decoupled ^{13}C-NMR spectrum, DEPT, HH COSY, HMQC, HMBC, NOE difference spectroscopy), using the recently developed computer program LUCY. Details on this program have been published elsewhere.[2] The major innovation of LUCY is the use of the valuable but ambiguous HMBC data in an initial computation step, greatly decreasing the number of solution compounds needed to be submitted to, for example, a database search.

The HREIMS of eupatoriochromene (**1**) showed a molecular ion peak at *m/z* 218.0946, which is consistent with the molecular formula $C_{13}H_{14}O_3$. This list of atoms was used as a starting point by the program LUCY. The ^{13}C-NMR spectrum displayed 12 signals, with one of them (28.63 ppm) showing double intensity. The DEPT spectra (DEPT-90 and DEPT-135) gave rise to the existence of six quaternary carbon atoms, four methine groups, and three methyl groups. The CH balance, showing 13 protons, revealed the existence of a hydroxyl group. As a result of this CH analysis, a list of CH fragments (DEPT-results) was given to the program. Further, the hybridizations of all non-hydrogen atoms were estimated on the basis of the carbon chemical shift. LUCY features separate input sheets for HMQC (or any other experiment revealing $^1J_{CH}$ couplings), HMBC (or any other experiment revealing $^2J_{CH}$ or $^3J_{CH}$ couplings, like the conventionally detected CH long-range HETCOR or the CH COLOC experiment) and HH COSY data.

Table 1. NMR Data of Eupatoriochromene (**1**) (500 MHz, 25 °C, $CDCl_3$)

carbon	δ_C	CH_n	δ_H ($^1J_{CH}$)	δ_H (long range)
2	78.0	C		6.27, 5.57, 1.43
3	129.0	CH	5.57	1.43
4	121.1	CH	6.27	7.30
4a	113.7	C		7.30, 6.31, 6.27, 5.57
5	128.7	CH	7.30	6.27
6	114.0	C		12.7[a]
7	165.2	C		12.7[a], 7.30, 6.31
8	104.6	CH	6.31	12.7[a]
8a	160.4	C		7.30, 6.31, 6.27
9/10	28.6	CH_3	1.43	5.57
11	202.5	C		7.30, 2.53
12	26.3	CH_3	2.53	

[a] Proton shift of attached OH group.

The 12 carbon and the six hydrogen shifts (Table 1) were entered into the HMQC data sheet, simultaneously correlating these by one-bond couplings. While this table allows for numerical input, the HMBC sheet is prepared by the program as a clickable map, using a knowledge of the HMQC table. Here, the 18 HMBC cross peaks of the eupatoriochromene data were inserted simply by mouse click. The cross peaks were interpreted as $^2J_{CH}$ and $^3J_{CH}$ couplings. The HH COSY data sheet allowed for the assignment of the $^3J_{HH}$ correlation between the protons at δ_H 5.57 and δ_H 6.27. Having started the calculation, the program prepared a list of CH fragments using a combination of HMQC and HH COSY data to establish C−C-bonds. It then exhaustively computed all possible interpretations of the HMBC cross peaks as two- or three-bond couplings. The result of the calculation for eupatoriochromene (**1**), which revealed two structures consistent with the input data, is shown in Figure 1. LUCY's structure output window only shows the connectivity of atoms, not the bond order. Therefore, we have prepared Figure 2 to summarize the result of the structure elucidation and to give the complete assignment of the NMR data, which is shown in Table 1. The second output structure can easily be excluded, e.g., by a subsequent shift calculation and comparison with the input data.

The procedure leading to the structural identification of preconene II (**2**) is to a great extent equivalent to the one described above for eupatoriochromene (**1**). In this case, the mass spectrometric data were not of high resolution. We inferred a molecular formula of $C_{13}H_{16}O_3$ from the molecular ion peak at *m/z* 220.1, taking into account 13 carbon atoms, 16 hydrogen atoms, and at

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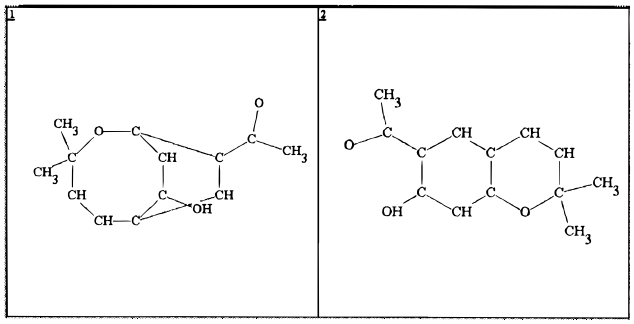

Figure 1. Screen shot of the LUCY structure display. Results of the eupatoriochromene (**1**) calculation. Only connectivity is shown, no bond orders.

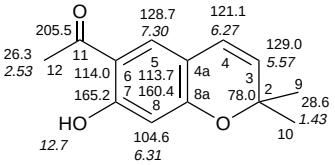

Figure 2. Structure of eupatoriochromene (**1**) (NMR data: 500 MHz, 25 °C, $CDCl_3$).

Table 2. NMR Data of Preconene II (**2**) (500 MHz, 25 °C, $CDCl_3$)

carbon	δ_C	CH_n	δ_H ($^1J_{CH}$)	δ_H (long range)
2	76.4	C		6.26, 5.50, 1.44
3	128.6	CH	5.50	1.44
4	122.4	CH	6.26	6.55
4a	113.4	C		6.44, 6.26, 5.50
5	110.0	CH	6.44	
6	143.5	C		3.85, 6.55, 6.44
7	150.0	C		3.86, 6.55, 6.44
8	101.4	CH	6.55	
8a	147.6	C		6.55, 6.44, 6.26
9/10	28.1	CH_3	1.44	5.50
11	56.9	CH_3	3.85	
12	56.3	CH_3	3.86	

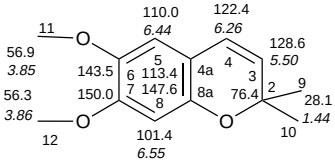

Figure 3. Structure of preconene II (**2**) (NMR data: 500 MHz, 25 °C, $CDCl_3$).

least two oxygen atoms that can be derived from the broad-band decoupled ^{13}C-NMR and the DEPT spectra. The NMR data are summarized in Table 2. The structure elucidation revealed only one possible structure, shown in Figure 3.

Eupatoriochromene (**1**) has been isolated from other *Calea* species before but not from *C. serrata*.[3−5] Preconene II (**2**) has not been described yet as a natural constituent in *Calea* species.

In conclusion, we have presented the semiautomatic structure elucidation of two compounds, eupatoriochromene (**1**) and preconene II (**2**), by a recently published program LUCY, using EIMS, broad-band-decoupled ^{13}C-NMR, DEPT, HMQC, HMBC, and HH COSY experiments. The structures have been elucidated by the program in the very short time of less than 30 s each. We believe that the routine application of this program in phytochemical analysis could greatly facilitate compound structure elucidation, especially because sophisticated techniques like gradient-enhanced HMQC and HMBC have shortened the recording time for the necessary NMR experiments remarkably.

Experimental Section

General Experimental Procedures. Automatic, preparative chromatography was performed on a Chromatotron Model 7924T (Harrison Research, Palo Alto, CA). NMR spectra were measured on a Bruker DRX 500 NMR spectrometer (Karlsruhe, Germany) using standard pulse programs provided by the manufacturer. Mass spectra were measured on an MS 902 mass spectrometer (A.E.I. Manchester, U.K.). The program LUCY was used under MS WINDOWS 95 on a standard IBM compatible PC with a 100-MHz Intel Pentium processor.

Plant Material. Aerial parts of *C. serrata* were collected from Morro Santana, Porto Alegre/RS, Brazil, by M. Sobral in August 1995. A voucher specimen (ICN 8294) is deposited in the Herbarium of the Faculty of Botany, UFRGS, Porto Alegre/RS, Brazil.

Extraction and Isolation. The chromene derivatives were isolated by drying 78.8 g of the aerial parts of *C. serrata*, finely powdering the material, and extracting them with $CHCl_3$. The evaporated extract was dissolved in EtOH and 4% of $Pb(Ac)_2$ was added to precipitate chlorophylls and other impurities. Filtration and further extraction with $CHCl_3$ yielded 620.3 mg of a crude extract. This extract was separated by automatic, preparative chromatography over Si gel [CH_2Cl_2−Et_2O (4:1)] using a chromatotron, giving 23 mg of the yellow, crystalline eupatoriochromene (**1**) and 10 mg of yellow, amorphous preconene II (**2**).

Eupatoriochromene (1): yellow needles from CH_2Cl_2−Et_2O; mp 80 °C; ^{1}H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 1; HREIMS *m/z* 218.0946, calcd for $C_{13}H_{14}O_3$ 218.0943.

Preconene II (2): yellow amorphous powder from CH_2Cl_2−Et_2O; mp 48 °C; ^{1}H NMR ($CDCl_3$), see Table 2; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 220.1, calcd for $C_{13}H_{16}O_3$ 220.1099.

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References and Notes

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