ORGANIC LETTERS

1999
Vol. 1, No. 12
1897−1900

A New Cyclohexadecane Derivative from *Trixis vauthieri* DC (Asteraceae)

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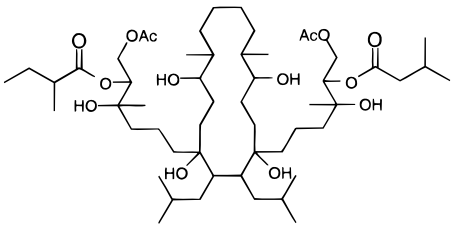
Received September 14, 1999

ABSTRACT



The dichloromethane–methanol extract from the fresh leaves of *Trixis vauthieri* DC (Asteraceae) afforded trixol, a new cyclohexadecane derivative. The structural elucidation of this new compound, with a novel skeleton, was based on NMR studies of the natural product and its derivatives.

Asteraceae is a mega-family of plants with approximately 25 000 species. A plethora of secondary metabolites of different chemical classes have been found in this family, including polyacetylenes, sesquiterpene lactones, essential oils, alkaloids, and flavonoids. In a biological screening of plants from the Brazilian savanna ("cerrado") we found that extracts from *Trixis vauthieri* (Asteraceae) were able to kill the protozoan parasite *Trypanosoma cruzi* present in the blood of experimentally infected mice.[1] This flagellated protozoan can infect man, provoking Chagas' disease (American trypanosomiasis), a severe condition that afflicts about 20 million people in Central and South America.[2] *T. vauthieri* was previously subjected to a phytochemical study by Bohlmann and co-workers who isolated 24 compounds from roots and aerial parts, mainly flavonoids and terpenoids.[3] Fresh leaves of *T. vauthieri* were collected in the vicinities of Belo Horizonte and extracted with (1:1) dichloromethane−methanol. After several fractionation steps, this extract yielded several flavonoids, two of them with trypanocidal activity.[4] Continuing this work, another trypanocidal fraction afforded a pure compound (**1**) isolated as a clear, viscous oil (0.06% yield from fresh leaves). This substance showed no UV absorption above 210 nm, indicating a lack of chromophores. Its IR spectrum showed signals corresponding to ν_{OH} (3400 cm^{-1}), ν_{CH} (2930 cm^{-1}), $\nu_{C=O}$ (1750 cm^{-1}), and ν_{C-O} (1250 cm^{-1}), indicating an aliphatic hydroxylated ester.

Analysis of the ^{13}C, ^{1}H, and DEPT ^{13}C NMR spectra of **1** allowed us to estimate the molecular formula $C_{54}H_{100}O_{16}$ (MW of 973 Da) for this natural product. The FABMS in positive ion mode showed a quasimolecular ion peak at 978 Da, attributed to $[M + Na - H_2O]^+$, corroborating the proposed molecular formula. The hydrogenation index corresponds to five unsaturations, four of them due to carbonyl groups, as suggested by the ^{13}C NMR spectrum. As no other

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(1) Zani, C. L.; Chaves, P. P. G.; Queiroz, R.; Mendes, N. M.; Oliveira, A. B.; Cardoso, J. E.; Anjos, A. M. G.; Grandi, T. S. *Phytomed.* **1995**, *2*, 47−50.
(2) WHO *Tropical Disease Research*; World Health Organization: Geneva, 1993; p 134.
(3) Bohlmann, F.; Suwita, A.; Jakupovic, J.; King, R. M.; Robinson, H. *Phytochem.* **1981**, *20*, 1649−1655.
(4) Ribeiro, A.; Pilo-Veloso, D.; Romanha, A. J. and Zani, C. L. *J. Nat. Prod.* **1997**, 60, 836−8.

10.1021/ol991045b CCC: $18.00
Published on Web 11/19/1999

signal due to sp^2 carbon was observed in the NMR spectra, the remaining unsaturation was attributed to a ring. The ^1H NMR spectrum of **1** showed signals only in the region of aliphatic hydrogens, between δ 0.80 and 5.20, showing several overlapped signals between δ 1.00 and 1.70. In the following discussion the arbitrary numbering shown in Figure 1 will be adopted. Using the methyl signals as starting points,

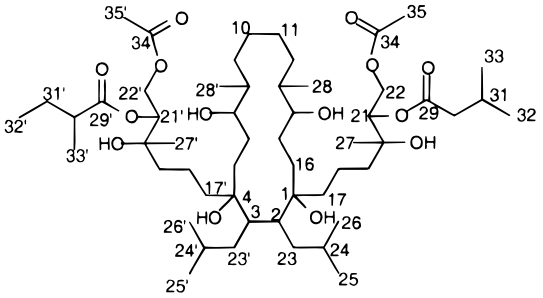


Figure 1. Numbering of carbons in trixol.

it was possible to deduce the presence of two methyl groups linked to tertiary hydroxylated carbons (δ 1.18, s, H-27 and H-27′). Also, the region δ 0.84−1.00 contains the signals of methyl groups belonging to two isobutyl, one isovaleroyl, and one 2-methylbutyroyl (Figure 2).

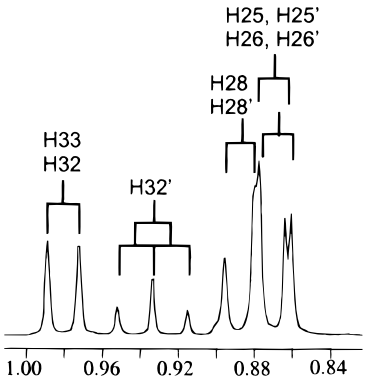


Figure 2. Expansion (δ 0.84−1.00) of the ^1H NMR spectrum (400 MHz, $CDCl_3$) of **1**.

The connectivity of the oxygenated carbons C-21 (δ 75.85) and C-22 (δ 63.63) and the corresponding C-21′ (δ 75.57) and C-22′ (δ 63.61) was established using HH-COSY and HMQC data. The protons connected to these carbons generated totally or partially superimposed signals in the ^1H NMR spectrum (Figure 3).

These carbons harbor the ester groups, and the relative positions of the substituents were defined by the long-range couplings, observed in the HMBC experiments, between their carbonyl carbons and the protons H-21 and H-22. In this way the acetyloxy groups were positioned at C-22 and C-22′ while isovaleroyloxy and 2-methylbutyroyloxy were positioned at C-21 and C-21′, respectively. HMBC correlations, shown in Figure 3, allowed the connection of these fragments to tertiary hydroxylated carbons C-20 and C-20′, respectively.

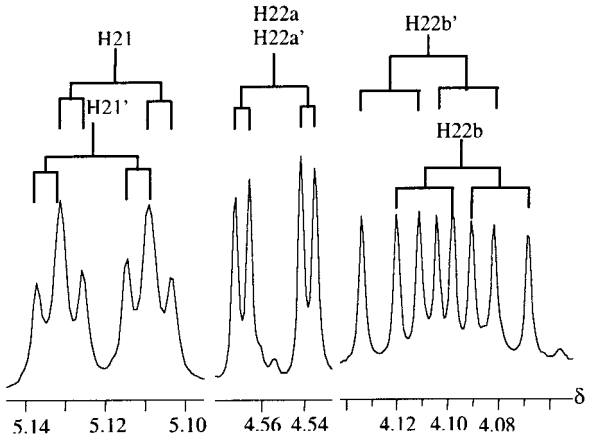
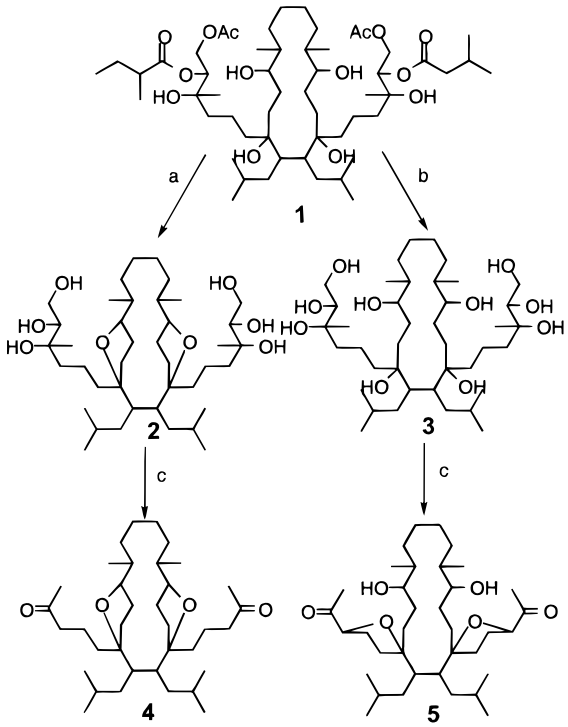


Figure 3. Expanded regions of ^1H NMR spectrum of trixol ($CDCl_3$, 400 MHz).

The core of the molecule contains 6 CH_3, 16 CH_2, 6 CH, 2 CH−O, and 2 C−O with one unsaturation (a ring). Their protons signals were highly overlapped in the ^1H NMR spectra, and the structural elucidation of this part of the molecule required the preparation of several derivatives (Scheme 1) and the extensive use of 2D NMR experiments.

Scheme 1



a. HCl 4N, 100 °C, 1h; b. MeONa, 25°C, 1h; c. HIO_4, MeOH-H_2O (75:25), 25°C, 1h.

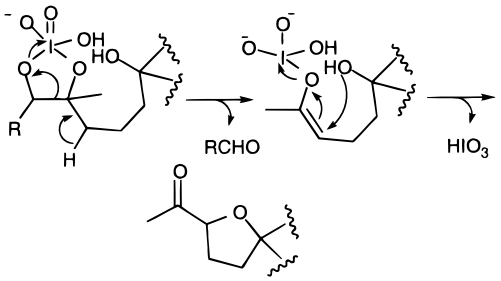
Table 1. NMR Data for Trixol

position	δ^{13}C (DEPT)	δ ^{1}H	$^2J_{C-H}$ (HMBC)	$^3J_{C-H}$ (HMBC)
1	72.92 (C)		H17; H16a and b; H2	
2, 3	27.65 (CH)	1.18 m		H16a and b; H5a′ and b
4	72.92 (C)		H17′; H16a′ and b; H3	
5	38.97 (CH_2)	1.68 m, 1.52 m		H7
6	29.08 (CH_2)	1.50 m	H7′; H5a and b	
7	76.18 (CH)	3.46 ddd (3.7, 4.0, 8.2)	H8; H6	H5a and b; H9a and b; H28′
8	39.03 (CH)	1.50 m	H7	
9	33.87 (CH_2)	1.40, 1.16 m	H8′	H10a; H10b; H28′
10	25.50 (CH_2)	1.38 m, 1.25 m	H9a and b	H8
11	25.50 (CH_2)	1.38 m, 1.25 m	H12a and b	H13
12	33.87 (CH_2)	1.40 m, 1.16 m	H13	H11a; H11b; H28
13	39.03 (CH)	1.50 m	H14	
14	76.18 (CH)	3.46 ddd (3.7, 4.0, 8.2)	H13; H15	H16a and b;H12a and b;H28
15	29.08 (CH_2)	1.50 m	H14; H16a and 16b	
16	38.97 (CH_2)	1.68 e 1.52 m		H14′
17	42.23 (CH_2)	1.48 m		
17′	42.23 (CH_2)	1.48 m		
18	17.83 (CH_2)	1.48 m		
18′	17.80 (CH_2)	1.48 m		
19	39.67 (CH_2)	1.45m, 1.20 m		
19′	39.58 (CH_2)	1.45m, 1.20 m		
20	73.56 (C)		H21	H22a; H22b
20′	73.53 (C)		H21′	H22a′; H22b′
21	75.85 (CH)	5.12 dd (2.3, 8.9)	H22a; H22b	H27
21′	75.57 (CH)	5.13 dd (2.3, 8.9)	H22a′; H22b′	H27′
22	63.63 (CH_2)	H22a 4.55 dd (2.3, 11.9)	H22b, H21	
		H22b 4.09 dd (8,9, 11.9)	H22a, H21	
22′	63.61 (CH_2)	H22a′ 4.55 dd (2.3, 11.9)	H22b′, H21′	
		H22b′ 4.11 dd (8,9, 11.9)	H22a′, H21′	
23, 23′	39.67 (CH_2)	1.18 m	H24	H25; H26; H25′; H26′
24, 24′	28.34 (CH)	1.54 m	H23;H25;H26,H23′;H25′;H26′	
25, 25′	23.11 (CH_3)	0.87 d (6.7)	H24; H24′	H26; H23; H26′; H23′
26, 26′	22.96 (CH_3)	0.86 d (6.4)	H24; H24′	H25; H23; H25′; H23′
27	23.65 (CH_3)	1.18 m	H21	
27′	23.62 (CH_3)	1.18 m	H21′	
28	14.31 (CH_3)	0.88 d (6.6)		H7′, H14
29	172.97 (C)		H30	H21; H31
29′	176.60 (C)		H30′	H21′; H31′
30	43.91 (CH_2)	2.25 m	H31	
30′	41.67 (CH)	2.45 m	H31a′; H31b′	
31	26.13 (CH)	2.15 m	H30; H33; H32	
31′	27.13 (CH_2)	1.70 e 1.55 m	H32′	H33′
32	22.69 (CH_3)	0.98 d (6.6)	H31	H30
32′	11.92 (CH_3)	0.93 d (7.4)	H31a′; H31b′	H30′
33	22.75 (CH_3)	0.98 d (6.6)	H30	H31
33′	17.18 (CH_3)	1.17 m	H30′	H31a′; 31b′
34	171.41 (C)		H35; H21	H22a; H22b
34′	171.39 (C)		H35′; H21′	H22a′; H22b′
35	21.21 (CH_3)	2.01 s		
35′	21.20 (CH_3)	2.00 s		

In this way we were able to discern the presence of a symmetric core substituted by the two slightly different ester groups just discussed. This hypothesis was confirmed by methanolysis of **1** that produced symmetric polyol derivative **3** (Scheme 1) showing only half of the signals in the ^{13}C NMR spectrum.

During the preparation of the derivatives, some reaction conditions allowed internal cyclizations yielding mixtures of isomers. Thus, the production of **2** as the major compound certainly involves a stabilized tertiary carbocation. During the oxidative cleavage of **3** using HIO_4, the formation of **5** was rationalized by the reaction mechanism shown in Scheme 2. These derivatives now contained carbonyls and cyclic ethers that altered some chemical shifts, allowing us to

Scheme 2



confirm the correlations already observed for the natural product (Figure 4).

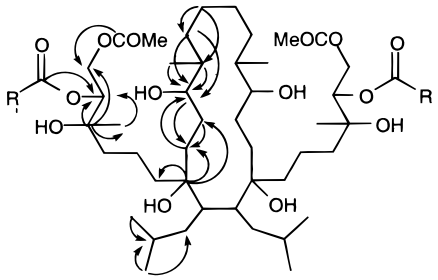


Figure 4. Relevant correlations observed in the HMBC spectra.

Because of the severe overlap of the signals, the connections C-1 to C-20 and C-4 to C-20′ were established by analysis of the NMR data of derivatives **4** and **5**. In **4**, the carbonyl at C-20 (δ 209.63) shows clear correlation with H-18 (δ 1.63), H-19 (δ 2.44), and the methyl protons at C-21 (δ 2.13). Correlation between C-1 (δ 81.86) and the methylene H-17 (δ 1.45) could also be observed. In the same way, the attachment of the isobutyl groups to C-2 and C-3 was confirmed by the HH-COSY contour map of **5**, for this shows a clear correlation of H-2 with H-23 and of H-3 with H-23′.

The fragment C-3−C-10 and the exact location of its substituents were deduced with extensive use of 2D NMR experiments, including TOCSY. All carbons in this half of the molecule, as deduced by the NMR data, have the necessary number of substituents, except for C-3 and C-10. We concluded that these carbons must be connected to their symmetrical counterparts, C-2 and C-11, respectively, on the other half of the molecule, to form the expected ring system.

In conclusion, except for the stereochemistry, complete structural elucidation with NMR signal attribution to every atom was possible for the natural product trixol (**1**). After allowing for the expected influences of the chemical changes that were made, all the shifts measured for the derivatives (**2**−**5**) are fully consistent with those of trixol shown in Table 1. To the best of our knowledge this is the first time that a compound with this skeleton has been characterized. The biosynthesis, distribution, and function of this compound in the plant are not known. Studies to define the configuration of each chiral center and to prepare crystalline derivatives to allow for X-ray crystallography are underway.

Acknowledgment. We thank Prof. G. A. Cordell (UIC, Chicago) for the FAB MS spectrum and CNPq, PRONEX, and FIOCRUZ for financial support and fellowships.

Supporting Information Available: NMR data table for all trixol derivatives (**2**—**5**). This material is available free of charge via the Internet at http://pubs.acs.org.

OL991045B