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THE STRUCTURE OF ZAPOTERIN(2, 3)

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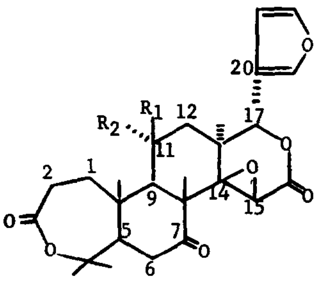
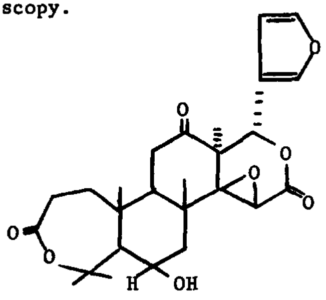
The isolation of zapoterin from the seeds of the Mexican tree *Casimiroa edulis* Llave *et* Lex (Rutaceae) has been described and an empirical formula $C_{19}H_{24}O_6$ claimed.(5) However, the mass spectrum revealed a molecular ion (M+) m/e 470 in agreement with an empirical formula $C_{26}H_{30}O_8$ and prompted further investigation.

Prominent in the NMR spectrum of zapoterin taken in hexadeuteriodimethylsulfoxide (d_6DMSO) were five quaternary methyl groups and multiplet low-field resonances at δ6.48 (one proton) and 7.68 p.p.m. (two protons), strongly suggestive of a limonoid triterpene incorporating a furan ring for which class a comprehensive review of NMR data was conveniently available.(6) The close relationship of zapoterin and obacunone (Ia, isolated earlier with zapoterin but named casimirolid)(7) became evident from comparative NMR spectroscopy.



I(a) R_1=R_2=H
(b) R_1=OH, R_2=H
(c) R_1=OAc, R_2=H
(d) R_1, R_2= =O



II

Sharp one-proton singlets for 15α and 17β-protons, and an AX quartet for the protons of the cis-disubstituted αβ-unsaturated lactone in ring A constitute useful supporting evidence. An AMX pattern at 2.29, 2.67, and 3.13 p.p.m. was assigned to the ketone system $-\overset{|}{C}H-CH_2\overset{|}{C}=O$ ($J_{gem.}$ 13, $J_{vic.}$ 13 and 4.5 c.p.s.). The absence of other couplings (>1 c.p.s.) for these three protons was compatible with, though not proof of, the presence of two fully substituted carbon atoms alpha to the methine carbon of this syste

The moiety $-\overset{|}{C}H\cdot CHOH\cdot CH_2-$, with both terminal carbons bearing most probably only fully substituted carbon as other substituents, was concluded from the following. An hydroxyl one-proton doublet centered on 5.02 p.p.m. (J = 4 c.p.s.) was shown (double resonance and D_2O exchange) to be coupled to the one-proton multiple resonance (C<u>H</u>OH) at 4.55 p.p.m., in turn coupled with the broad singlet one-proton resonance at 1.96 and to some unresolved obscured resonance <u>ca</u>. 1.4 - 1.8 p.p.m. No other strong couplings of these four protons was detected. From the above observations, and arguments developed below, structures I(b) or II for zapoterin appeared reason-

The NMR spectrum of "isozapoterin" (an alleged isomer of zapoterin)$^{(5)}$ was examined next in $CDCl_3$ owing to solubility considerations and its identity as zapoterin acetate I(c) deduced, together with structure I(b) for zapoterin. "Isozapoterin" shows a 3-proton acetate methyl singlet resonance at 2.19 p.p.m. (d_6DMSO solution) and a C<u>H</u>OAc resonance 1.08 p.p.m. downfield from the C<u>H</u>OH NMR signal frequency for zapoterin. In $CDCl_3$ the acetate showed resonances assignable to the 15α- and 17β-protons at 3.74 and 5.44 p.p.m. respectively, in agreement with placing a keto group at C-7$^{(8)}$ and indicate structure I(c) for this compound.

Spectra of zapoterin and its derivatives in a variety of deuterated solvents were then measured in an attempt to resolve the complex AMKY system (C-11α, C-9, C-12 geminal protons respectively). Spectra taken in d_5-pyridine permitted a first order analysis and the following coupling constants were measured: $J_{12\alpha,12\beta}$ 15, $J_{11\alpha,12\alpha}$ 5.5, $J_{11\alpha,12\beta}$ 1, and $J_{11\alpha,9}$ 1 c.p.s., values in fair agreement with those calculated on the basis of dihedral angle measurements from models. The very low field position of two of the quaternary methyl group resonances for zapoterin, relative to obacunone,$^{(9)}$ indicates for the former a 1,3-diaxial relationship between an 11β-hydroxyl group and the two methyl groups at C-8 and C-10.$^{(10)}$ The spectrum of zapoterone I(d)$^{(11)}$ taken in d_5-pyridine supplied firm evidence for structure I(b) for zapoterin. A sharp AM quartet assigned to the C-12 protons resonated at 2.49 and 2.79 p.p.m. and the C_9-H as a slightly broadened singlet at 3.71 p.p.m. Spectral data are summarized in Table I.

TABLE I

NMR Spectra of Zapoterin Derivatives Taken in d$_5$Pyridine

Compound	Me Groups	H-1	H-2	H-5α	H-6α	H-6β	H-9	H-11α	H-12α	H-12β	H-15	H-17	alpha furan (2H)	beta furan (H)
Obacunone	1.17 1.25 1.29 1.43 1.43	6.64 d 11	6.10 d 11	2.77 dd 13,4	2.35 dd 13,4	3.02 dd 13,13	complex multiple resonance ~1.3-1.9 p.p.m.				4.04 s	5.62 s	7.55 m	6.43 m
Zapoterin	1.22 1.32 1.50 1.83 2.00	6.92 d 12	6.15 d 12	2.86 dd 13,4	2.48 dd 13,4	3.20 dd 13,13	2.23 bs	4.95 bd 6	2.19 d 15,6	2.10 d 15	4.22 s	5.83 s	7.55 m	6.43 m
Zapoterone	1.28 1.40 1.43 1.45 1.96	6.83 d 12	6.08 d 12	2.95 dd 13,4	2.55 dd 13,4	3.20 dd 13,13	3.70 bs	-	2.80 d 19	2.47 d 19	4.42 s	5.78	7.62 7.74 m	6.53 m

s = singlet
d = doublet
dd = double doublet
bs = broadened singlet
m = multiplet

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