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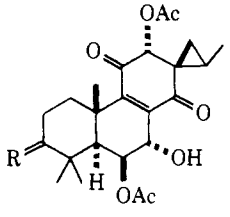
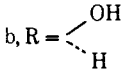
Structure and Stereochemistry of Cyclobutatusin, a Diterpenoid Containing a Four-Membered Ring

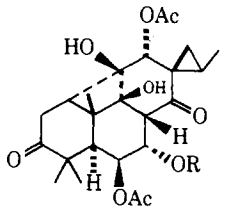
Sir:

Biological screening of plants from Brazilian flora[1] led to an investigation of the bitter principles contained in the leaves of *Coleus barbathus* (*Labiatae*) which are currently used in popular medicine as a stomach aid.[2] This study has resulted in the isolation and characterization of barbatusin (Ia), a diterpene containing a spirocyclopropane ring attached at C-13.[3] We wish to report now the structures of two of the minor constituents identified in this plant. The more interesting compound is cyclobutatusin (IIa), a novel diterpenoid which retains the basic ring skeleton of barbatusin, but has the outstanding new feature of a four-membered ring formed by a bond between C-1 and C-11.



Ia, R = O

b, R = 



IIa, R = H

b, R = COC_6H_4Br

Chromatography on silica gel of an acetone extract of the plant material afforded, upon elution with an acetone–chloroform mixture, cyclobutatusin (0.008%), mp 196–200°, $C_{24}H_{32}O_9$ (M^+ 464). No major uv absorption maximum above 210 nm was observed, whereas the ir spectrum (KBr) indicated the presence of hydroxyl, keto, and ester functions (3600, 1710, 1380, 1200 cm^{-1}). The nmr spectrum ($CDCl_3$, ppm) indicated three tertiary and one secondary *C*-methyl group signals (s at 1.12, 1.13, and 1.34; d at 1.19, J = 6.5 Hz) for the 18-, 19-, 20-, and 17-CH_3, respectively; two acetyl functions at 2.02 (3 H, s, 6β-OAc) and 2.11 (3 H, s, 12α-OAc), two C*H*OAc protons at 5.54 (d of d, $J_{5,6}$ = 4.8 and $J_{6,7}$ = 6.4 Hz) and at 5.58 (s) for the 6α-H and 12β-H; the complex signal pattern of the three hydroxyl groups (br signals at 4.3 and 4.49 with $W_{1/2}$ = 22 and 14 Hz) collapsed following deuterium exchange (with D_2O) into a simple d of d at 3.97 ppm ($J_{6,7}$ = 6.4 and $J_{7,8}$ = 4 Hz, assigned to the 7β-H). Cyclobutatusin readily formed a mono-*p*-bromobenzoyl ester (IIb), $C_{31}H_{35}O_{10}Br$, mp 286–288° (M^+ 646 and 648), a fact which pointed out that two of the hydroxyl groups were tertiary. From the detailed spectroscopic analyses and from biogenetic considerations, a structure related to barbatusin (Ia) was strongly suggested for cyclobutatusin. At this stage, recourse was made to X-ray analysis for determination of the structure.

A colorless rectangular plate-shaped crystal of the *p*-bromobenzoyl ester (IIb) obtained by crystallization from benzene was used to deduce the structure and stereochemistry of cyclobutatusin by X-ray analysis. Crystal data are: $C_{31}H_{35}O_{10}Br$; mol wt 647.5; orthorhombic; a = 14.091 (3), b = 15.268 (3), c = 14.018 (3) Å; V = 3016 Å^3; ρ_{obsd} = 1.44 g cm^{-3}; Z = 4; ρ_{calcd} = 1.43 g cm^{-3}; $F(000)$ = 1344; μ (Cu Kα) = 25.6 cm^{-1}; space group $P2_12_12_1$. A total of 2241 nonzero reflections out to 2θ = 130° was measured on a Picker FACS-1 diffractometer using Cu Kα radiation.[4] The structure was solved by the heavy atom method and refined by full-matrix least-squares methods with anisotropic thermal parameters for all nonhydrogen atoms. Hydrogen atoms were located at the most probable positions based on two successive difference maps and were included in the structure factor calculations but were not refined. The absolute configuration of II was assumed to be related to that of barbatusin. The final R factor was 0.069. A stereoscopic drawing of the molecule of IIb is shown in Figure 1.

The striking geometrical features of the molecule are the highly constrained skeleton and the overall compactness. If one neglects the *p*-bromobenzoyl side group, the molecule IIb assumes a bowl shape with the two adjacent hydroxyl groups at C-9 and C-11 exposed on the surface of the bottom of the bowl. The cyclobutane ring is not planar but has a dihedral angle of 19.0° between the planes defined by C-11, C-1, and C-10 and by C-11, C-9, and C-10.

During the chromatographic fractionations, a second minor compound was isolated and was assigned the structure Ib (3β-hydroxy-3-deoxobarbatusin (0.014%)) on the following grounds: $C_{24}H_{32}O_8$ (M^+ 448); uv max (EtOH) 235 nm (ε 18,273); ir (KBr) 3600, 1730, 1700, 1680, 1604, 1370, 1230 cm^{-1}. The nmr spectrum ($CDCl_3$, ppm) was particularly informative in that it revealed the presence of three tertiary *C*-methyl groups, signals at 0.99 (3 H, s, 18-CH_3),[5] 1.14 (3 H, s, 19-CH_3), and 1.66 (3 H, s, 20-CH_3), a secondary *C*-methyl group at 1.1 (3 H, d, $J_{15,17}$ = 6.5 Hz, 17-CH_3), two acetyl functions at 2.02 (3 H, s, 6β-OAc), 2.06 (3 H, s, 12α-OAc), two hydroxyl groups, broad signal centered at 2.80 (2 H, $W_{1/2}$ = 22 Hz, 3β- and 7α-OH), one proton at 3.28 (d of d, $J_{3,2}$ = 7.0 and 9.0 Hz for 3α-H),[6] one

(1) C. M. Rosito, D. Erlich, M. Nakagawa, and R. Zelnik, *An. Acad. Brasil. Cienc.*, 43, 687 (1971).

(2) A preliminary antitumor screening of an extract of the leaves has shown no significant activity against L-1210 lymphoid leukemia in mice. The tests were performed under the auspices of the Cancer Chemotherapy National Screening Center, National Cancer Institute.

(3) A. H.-J. Wang, I. C. Paul, R. Zelnik, K. Mizuta, and D. Lavie, *J. Amer. Chem. Soc.*, 95, 598 (1973).

(4) During the course of data collection (~8 days), the intensities of the three standards fell off gradually and the orientation matrix had to be recalculated several times. At the end of data collection the standards had fallen to 83% of their initial values and the cell dimensions were remeasured and found to be a = 14.141 (5), b = 15.250 (6), and c = 14.075 (5) Å. These differences suggested that in a few of the unit cells the compound may have undergone some change under X-ray irradiation. However, no clear evidence to indicate the nature of this change could be obtained from our X-ray study. A linear scale factor was applied to correct the fall-off in the intensities.

(5) The numbering system used in the discussion of the nmr spectra of Ib is that of the abietane ring system, which differs somewhat from that used to describe the molecular geometry of IIb (Figure 1).

(6) The β (equatorial) configuration of the hydroxyl group was established on the basis of the coupling constants of the methine proton at C-3. The latter proton is shown to be axially oriented on the basis of the angle values derived from the Karplus equation.

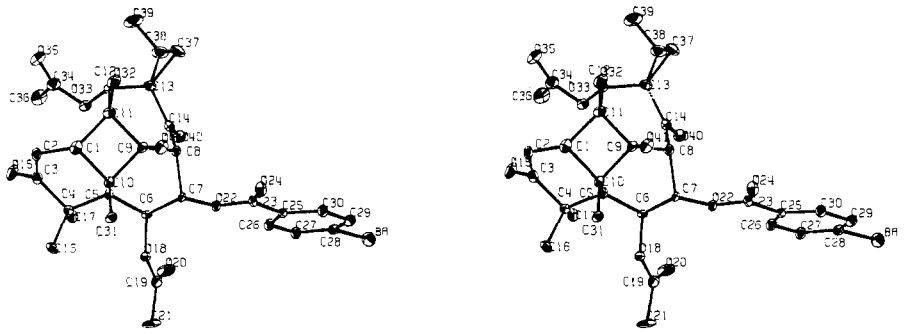


Figure 1. Stereoscopic view of the molecule IIb.

proton at 4.49 (d, $J_{7,6}$ = 2 Hz, 7β-H), one C*H*OAc proton at 4.85 (s, 12β-H), one C*H*OAc proton at 5.44 (t, $J_{6,7}$ = 2 Hz, $J_{5,6}$ = 0.5 Hz, 6α-H) (observed by decoupling experiments). The structural assignment of this compound was settled by careful oxidation with Jones reagent (60 min, 10°), whereupon barbatusin (Ia) was obtained in 80% yield.

Cyclobutatusin (IIa) and 3β-hydroxy-3-deoxobarbatusin (Ib) join the class of natural products with a cyclopropane ring, the biological and biogenetical importance of which is now fully recognized.[7] Although a number of monoterpenes and sesquiterpenes with a four-membered ring are known,[8] cyclobutatusin appears to be the first naturally occurring substance in the diterpenoid series with such a feature. The formation of cyclobutanol derivatives upon irradiation of steroids[9] and triterpenoids[10] has been extensively studied and our present findings raise the question of whether cyclobutatusin is part of a biogenetic sequence for quinonoid-type diterpenes[3] or whether it may be the product of a photochemically induced reaction of a barbatusin-type precursor. Investigations to resolve this question are now in progress. Pharmacological testing involving antitumor and antibacterial assays with barbatusin and cyclobutatusin are under way and will be reported in due course.[11]

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Supplementary Material Available. The final atomic coordinates for IIb will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-580.

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A Model for the Proton Transfer Stages of the Biological Transaminations and Isotopic Exchange Reactions of Amino Acids[1]

Sir:

Stereospecific transamination reactions are important to the biological elaboration of amino acids.[2] The enzyme-catalyzed reactions of eq 1 are stereospecific, and the proton (or isotope) transfer occurs intramolecularly.[3,4] Pyridoxal-containing enzymes catalyze isotopic exchange of the α hydrogens of L-amino acids with a high retention of configuration.[5] The reactions of eq 2 were stereospecific and occurred partially intramolecularly.[6] The starting imine underwent isotopic

(1) This work was supported by U. S. Public Health Service Research Grant No. GM 12640-09 from the Department of Health, Education and Welfare.

(2) (a) A. E. Braunstein and M. M. Shemyakin, *Biokhimiya*, **18**, 393 (1953); (b) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Amer. Chem. Soc.*, **76**, 648 (1954); (c) E. E. Snell in "Chemical and Biological Aspects of Pyridoxal Catalysis," P. M. Fasella, A. E. Braunstein, and A. Rossi-Fanelli, Ed., Macmillan Co., New York, N. Y., 1963, pp 1–12; (d) E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., "Pyridoxal Catalysis: Enzymes and Model Systems," Interscience, New York, N. Y., 1968.

(3) (a) H. C. Dunathan, L. Davis, P. G. Gray, and M. Kaplan, *Biochemistry*, **7**, 4532 (1968); (b) J. E. Ayling, H. C. Dunathan, and E. E. Snell, *ibid.*, **7**, 4537 (1968); (c) G. B. Bailey, T. Kusamrarm, and K. Vuttirej, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **29**, 857 (1970); (d) B. S. Sukhareva, H. C. Dunathan, and A. E. Braunstein, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **15**, 241 (1971); (e) P. Besmer and D. Arigoni, *Chimia*, **23**, 190 (1969).

(4) For a review of the mechanism, see W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 143–145.

(5) A. F. Thomas, "Deuterium Labeling in Organic Chemistry," Appleton-Century-Crofts, New York, N. Y., 1971, pp 428–430.

(6) (a) D. J. Cram and R. D. Guthrie, *J. Amer. Chem. Soc.*, **87**, 397 (1965); (b) R. D. Guthrie, D. A. Jaeger, W. Meister, and D. J. Cram, *ibid.*, **93**, 5137 (1971).

(7) A. Burger, *Progr. Drug Res.*, **15**, 227 (1971); C. D. Poulter, O. J. Muscio, C. J. Spillner, and R. G. Goodfellow, *J. Amer. Chem. Soc.*, **94**, 5921 (1972); J. H. Law, *Accounts Chem. Res.*, 4, 199 (1971).

(8) See, for example, T. K. Devon and A. I. Scott, "Handbook of Naturally Occurring Compounds, Vol. II: Terpenes," Academic Press, New York, N. Y., 1972.

(9) J. Iriarte, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **46**, 1599 (1963); E. Altenburger, H. Wehrly, and K. Schaffner, *ibid.*, **48**, 704 (1965).

(10) J. Fried and J. W. Brown, *Tetrahedron Lett.*, 1677 (1966); M. Mousseron-Canet and J. P. Chabaud, *Bull. Soc. Chim. Fr.*, 239 (1969); B. W. Finucane and J. B. Thomson, *J. Chem. Soc. C*, 1569 (1971).

(11) See paragraph at end of paper regarding supplementary material.