Tetrahedron Letters,Vol.23,No.8,pp 811-814,1982 0040-4039/82/080811-04$03.00/0
Printed in Great Britain

STRUCTURE OF PROTOVERATRINE C, A NEW ALKALOID FROM PROTOVERATRINE

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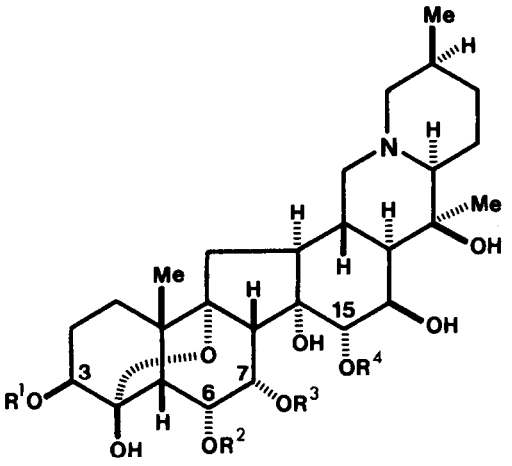
Abstract Single-crystal X-ray analysis has established that protoveratrine C, a third tetraester component of protoveratrine, is protoverine 3-(2'S,3'R)-2',3'-dihydroxy-2'-methylbutyrate 6,7-diacetate 15-(2'R)-methylbutyrate.

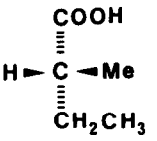
Use of crude veratrum extracts in the control of hypertension was first described by Baker in 1859,[1] but lack of reproducible results with these preparations considerably limited their therapeutic scope. First isolated from *veratrum album* and named by Salzberger,[2] the highly active alkaloid preparation protoveratrine became available in crystalline form some 50 years later.[3,4] This subsequently led to the introduction of protoveratrine into clinical practice for the treatment of certain types of hypertension.

In 1952-53, four groups[5] independently established that protoveratrine was not a single entity, but rather was a mixture of two very closely related ester alkaloids, protoveratrines A (1) and B (2). Clinical studies have demonstrated significant differences between these two alkaloids when administered orally.[6] Thus, (1) is a potent hypotensive agent with a narrow dosage range between hypotensive and undesirable emetic activity, whereas (2) has shown strong hypotensive activity of greater duration accompanied by reduced emetic potential.

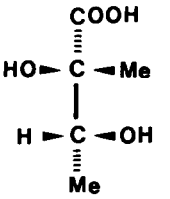
In their notable studies on veratrum alkaloids, Kupchan *et al.*[7] elucidated the structures of the parent alkamine protoverine (3) and of the tetraesters (1) and (2), save for the absolute configurations of the esterifying acids at C(3) and C(15). We have now found that protoveratrine,[8] long believed to be comprised of only these two tetraesters, contains in addition a third new protoverine tetraester which we have named protoveratrine C.

Protoveratrine C (4), a colorless crystalline solid[9] {m.p. 258-260 °C (dec.), $[\alpha]_D^{26}$ -6.6° ($CHCl_3$)}, was isolated in 4-5% yield by short column chromatography of protoveratrine.[10, 19] The alkaloid gave a molecular ion peak at 809, confirming the molecular formula $C_{41}H_{63}NO_{15}$ assigned





(6)



(7)

Compound	R^1	R^2	R^3	R^4
(1)	(+)-(2'S)-2'-hydroxy-2'-methylbutyryl	Ac	Ac	(-)-(2'R)-methylbutyryl
(2)	(+)-2',3'-dihydroxy-2'-methylbutyryl	Ac	Ac	(-)-(2'R)-methylbutyryl
(3)	H	H	H	H
(4)	(-)-(2'S,3'R)-2',3'-dihydroxy-2'-methylbutyryl	Ac	Ac	(-)-(2'R)-methylbutyryl
(5)	H	Ac	Ac	(-)-(2'R)-methylbutyryl

on the basis of elemental analysis.[11] Treatment of (4) with sodium periodate, followed by dilute ammonia work-up of the oxidation product, gave protoverine 15-(-)-2'-methylbutyrate 6,7-diacetate [desatrine (5)], identical in all respects with an authentic sample derived from (2). It was therefore evident that (2) and (4) differed only in the absolute configurations of the 2',3'-dihydroxy-2'-methylbutyrate ester substituents at C(3). The complete structure of (4) was established unequivocally by single-crystal X-ray analysis.

Crystals of (4) belong to the orthorhombic system, space group $P2_12_12_1$, with a = 14.120(7), b = 24.282(12), c = 12.769(9) Å, U = 4378 Å^3, Z = 4, D_c = 1.229 g cm^{-3}. The structure was solved from F_o and difference Fourier syntheses phased initially by atomic co-ordinates for the protoverine skeleton in crystals of isomorphous (1).[12] Least-squares adjustment of atomic positional[13] and thermal parameters converged to R = 0.067 over 3104 statistically significant reflections measured on an Enraf-Nonius CAD-3 automated diffractometer[14] (Ni-filtered Cu-K_α radiation, λ = 1.5418 Å; θ-2θ scans). A view of the solid-state conformation of (4) and the atom numbering scheme are shown in the Figure.

When the entire relative stereochemistry of (4), determined by the X-ray analysis, is considered in conjunction with the previously defined[15] absolute stereochemistry of (3), it yields (2'S,3'R) and (2'R) configurations, respectively, for the ester functions at C(3) and C(15). Thus, (4) is protoverine 3-(2'S,3'R)-2',3'-dihydroxy-2'-methylbutyrate 6,7-diacetate 15-(2'R)-2'-methylbutyrate. The results of the X-ray analysis of (4) [and (1)[12]] provide the first recorded proof of the absolute configuration of laevorotatory (2R)-methylbutyric acid (6) which is found in the C(15) ester linkage in (1), (2), and (4). The ester at C(3), derived from laevorotatory (2S,3R)-2,3-dihydroxy-2-methylbutyric acid (7),[16] also occurs in germitetrine and neogermbudine, two minor alkaloids derived from the alkamine germine.[17] The absolute configuration of the diastereomeric 2',3'-dihydroxy-2'-methylbutyrate ester present in (2) as well as germbudine has not yet been established; we shall discuss this point in detail in a later communication.

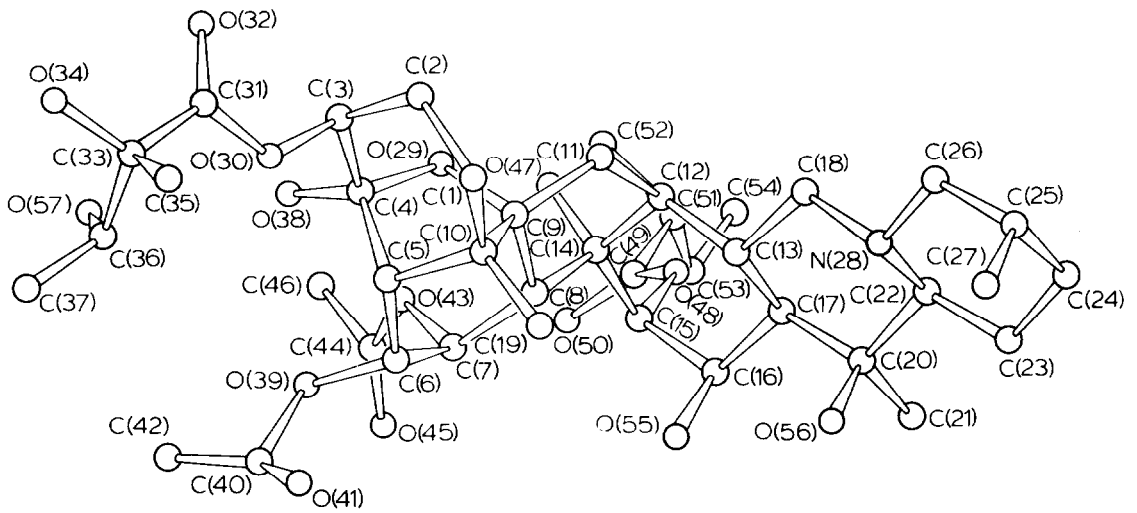


Figure. Atom numbering scheme and solid-state conformation of protoveratrine C (4)

Acknowledgements

We cordially thank Dr. John G. Topliss and Dr. Michael J. Green for their keen interest and encouragement. We should also like to thank Mr. R. Novotny, Mr. P. Bartner, Mrs. Bernice Evans and Mrs. Ailen Tseng for providing spectral and analytical data.

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8. (a) Protoveratrine used in this investigation, protobore®, was supplied by S. B. Penick and Co. who provided the following specifications for this material suitable for drug formulation: protoveratrine A (54.4%), protoveratrine B (42.4%), and non-protoveratrine (3.2%); (b) The names protoveratrine A and protoveratrine B were given to these compounds to indicate their being part of the recognized chemical entity "protoveratrine". See also ref. 5(C).
9. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, J = 7 Hz, $CH_2\underline{CH_3}$); 1.34 [3H, s, O.CO.C(OH).$\underline{CH_3}$]; 2.08 (3H, s, OCO$\underline{CH_3}$); 2.16 (3H, s, OCO$\underline{CH_3}$); 2.5 [1H, d, $J_{5,6}$ = 3 Hz, H(5)]; 3.1 [1H, d, $J_{7,8}$ = 5 Hz, H(8)]; 3.84 [1H, m, CH_3.C(OH)-$\underline{H}$]; 4.25 [1H, dd, $W_{\frac{1}{2}}$ = 8 Hz, H(16)]; ∿5.12 [1H, overlapping, H(3)]; 5.15 [1H, d, $J_{15,16}$ = 3 Hz, H(15)]; 5.6 [1H, dd, $J_{6,7}$ = 3 Hz, H(7)]; 6.02 [1H, J ∿3 Hz, H(6)].
10. (a) B. J. Hunt and W. Rigby, Chem. and Ind., 1868 (1969); (b) Silica gel GF: 5% (MeOH:NH_4OH; 9:1) in $CHCl_3$; R_f values: 0.6 (1); 0.33 (2), 0.44 (4).
11. Found: C, 60.9; H, 8.27; N, 1.56. Calc. for $C_{41}H_{63}NO_{15}$: C, 60.8; H, 7.78; N, 1.73.
12. A. T. McPhail, unpublished results.
13. Atomic co-ordinates for (4) have been deposited with the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.
14. For details, see: R. W. Miller and A. T. McPhail, J. Chem. Soc., Perkin Trans. 2, 1527 (1979).
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16. The dextrorotatory 2-hydroxy-2-methyl butyric acid in the ester linkage at C(3) in protoveratrine A, deacetylprotoveratrine A, germerine, and germitrine, and in the bitter principle glaucarubin has been proven earlier[18] to possess a (2S) configuration; the results[12] of a single crystal X-ray analysis of (1) are consistent with this assignment.
17. B. W. Christensen and A. Kjaer, Proc. Chem. Soc., 307 (1962).
18. B. W. Christensen and A. Kjaer, Acta Chem. Scand., 16, 2466 (1962).
19. For column chromatography (E. Merck, Silica Gel 60G), 40% acetone in chloroform was used as eluent. Protoveratrine C was isolated from middle fractions and recrystallized from CH_2Cl_2/n-Hexane.

(Received in USA 2 December 1981)