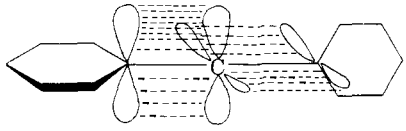
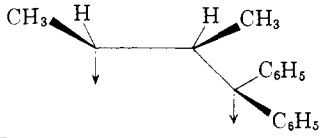
activity sequence. Also it has been reported that diphenylmethylene reacts with oxygen to produce benzophenone, and tetraethyl-*p*-phenylenediamine to give a blue Wurster's salt.[8]

These radical properties lead us to the assignment of a diradical (two electrons with parallel spins) structure to diphenylmethylene. Application of Hund's stabilization rule leads to the placement of these electrons in different orbitals which are nearly equivalent in stabilization. A rationalization consistent with these requirements involves a central sp carbon (orthogonal p-orbitals) and orthogonal aromatic nuclei. This rationalization has the virtue of permitting each benzene ring to interact with a different electron, the two benzene nuclei being insulated from one another, thus leading to a structure which might be described as two resonance stabilized benzyl radicals.



The spin conservation rules lead one to anticipate in the olefin addition reaction an open chain diradical intermediate of appreciable lifetime, permitting rotation about the single bond to compete with ring closure, and thus accounting for the nonstereospecific addition to *cis*- and *trans*-2-butene.[9]



(8) W. Kirmse, L. Horner and H. Hoffmann, *Ann.*, **614**, 19 (1958).

(9) Although it is not desirable to assign different names to the different spectroscopic states of a molecule, there might be sufficient reason for doing so with the triplet and singlet states of bivalent carbon. For the benefit of the chemist it is here suggested that all triplet states be given the traditional names of methylene derivatives and the name carbene be reserved for singlet states. Thus the name would convey the implication of radical or non-radical chemical properties. The authors welcome comments regarding this proposal.

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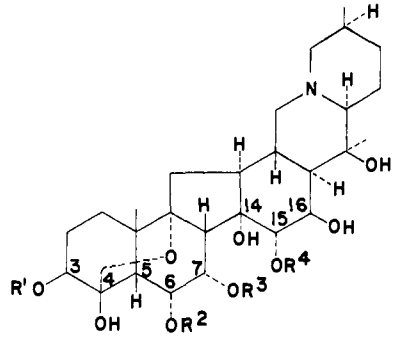
RECEIVED NOVEMBER 29, 1958

VERATRUM ALKALOIDS. XXVII. THE STRUCTURE OF PROTOVERATRINE A[1]

Sir:

Protoveratrine A[2] is a clinically useful hypotensive ester alkaloid.[3] Evidence is advanced herewith for assignment of structure I to protoveratrine A.

Alkaline hydrolysis[2c,d] of protoveratrine A has afforded the known alkamine protoverine[4] (II),[1] two mol. eq. of acetic acid, one mol. eq. of (*l*)-2-methylbutyric acid and one mol. eq. of (*d*)-2-hydroxy-2-methylbutyric acid. Protoveratrine A consumed 0.9 mol. eq. of chromic acid, an indication that the C_4 hydroxyl group is not acylated in the tetraester. The oxidation product, protoveratrone A, m.p. 221–223° dec., $[\alpha]^{25}D$ −97° (*c* 1.18, py.), on alkaline hydrolysis afforded an amorphous diosphenol with spectral properties identical with those of the diosphenol obtained from alkaline hydrolysis of 16-dehydroprotoverine 3,4,6,7,15-pentaacetate.[1] Thus, the C_{16} hydroxyl group is not acylated in protoveratrine A. Protoveratrine A readily formed a monoacetate, m.p. 249–250° dec., $[\alpha]^{22}D$ −52° (*c* 1.07, py.), and a mono-isobutyrate, m.p. 245–246° dec., $[\alpha]^{21}D$ −41° (*c* 1.36, py.). Protoveratrine A was obtained by methanolysis of the isobutyrate; this fact provides supporting evidence for a free C_{16} hydroxyl group in the naturally occurring tetraester (*cf.* ref. 1).



I, R^1 = HMB; R^2 = R^3 = Ac; R^4 = MB
II, R^1 = R^2 = R^3 = R^4 = H
III, R^1 = R^2 = R^3 = H; R^4 = MB
IV, R^1 = HMB; R^2 = R^3 = H; R^4 = MB
MB = (*l*)-2-methylbutyryl
HMB = (*d*)-2-hydroxy-2-methylbutyryl

Vigorous methanolysis of protoveratrine A resulted in loss of two acetyl groups. The resulting diester, protoverine mono-(*l*)-2-methylbutyrate mono-(*d*)-2-hydroxy-2-methylbutyrate, m.p. 203–205° dec., $[\alpha]^{23}D$ −19° (*c* 1.07, py.), consumed 0.9 mol. eq. of sodium periodate. The infrared spectrum of the amorphous oxidation product did *not* show absorption characteristic of the γ-lactone formed by periodate cleavage in Ring A of protoverine derivatives.[1] Furthermore, cyanometric titration[5] of the oxidation product indicated the presence of two aldehyde groups (from scission between C_6 and C_7). Thus the diester is a protoverine 3,15-diester and protoveratrine A has acetate groups at C_6 and C_7. Acetylation of the diester yielded protoveratrine A monoacetate.

A protoverine mono-(*l*)-2-methylbutyrate, m.p. 218–220° dec., $[\alpha]^{23}D$ −18° (*c* 0.97, py.), also was isolated from the methanolysis of protoveratrine A. This compound consumed 1.9 mol. eq. of sodium periodate, an indication that the (*l*)-2-methylbutyryl residue was attached to the C_{15} hydroxyl group. This was confirmed by acetylation to a tetraacetate, m.p. 262–263° dec., $[\alpha]^{23}D$ −46° (*c* 1.10, py.), shown to be protoverine 15-mono-(*l*)-2-methylbutyrate 3,6,7,16-tetraacetate as

(1) Part XXVI in the series: S. M. Kupchan, M. Neeman, C. I. Ayres, R. Hensler and S. Rajagopalan, *Chemistry and Industry*, 1626 (1958).

(2) (a) W. L. Glen, G. S. Myers, R. Barber, P. Morozovitch and G. A. Grant, *Nature*, **170**, 932 (1952); (b) M. W. Klohs, R. Arons, M. D. Draper, F. Keller, S. Koster, W. Malesh and F. J. Petracek, THIS JOURNAL, **74**, 5107 (1952); (c) H. A. Nash and R. M. Brooker, *ibid.*, **75**, 1942 (1953); (d) A. Stoll and E. Seebeck, *Helv. Chim Acta*, **36**, 718 (1953).

(3) O. Krayer in V. A. Drill, "Pharmacology in Medicine," McGraw-Hill Book Co., Inc., New York, N. Y., Second Edition, 1958, pp. 515–524.

(4) L. C. Craig and W. A. Jacobs, *J. Biol. Chem.*, **149**, 271 (1943).

(5) J. R. Dyer in David Glick "Methods of Biochemical Analysis," Interscience Publishers, Inc., New York, N. Y., Volume III, 1956, p. 132.

described. Protoverine 3,6,16-triacetate[1] on treatment with a limited amount of (*l*)-2-methylbutyryl chloride[6] afforded protoverine 15-(*l*)-2-methyl butyrate 3,6,16-triacetate, m.p. 234–235° dec., $[\alpha]^{28}D$ −4° (*c* 0.98, py.), which was stable toward sodium periodate but consumed 1.0 mol. eq. of chromic acid. Acetylation of the latter compound gave a pentaester identical with the product of acetylation of the monoester methanolysis product (III) from protoveratrine A. Thus the diester methanolysis product is IV, and protoveratrine A is protoverine 3-(*d*)-2-hydroxy-2-methylbutyrate 6,7-diacetate 15-(*l*)-2-methylbutyrate (I).[7,8]

(6) F. L. Weisenborn, J. W. Bolger, D. B. Rosen, L. T. Mann, Jr., L. Johnson and H. L. Holmes, THIS JOURNAL, **76**, 1792 (1954).

(7) Satisfactory analytical and spectral data were obtained for all the new compounds reported herein.

(8) We thank Dr. Harold A. Nash of the Pitman–Moore Company for a generous gift of protoveratrine A, and the National Institutes of Health (H-2275(C3)) and the Wisconsin Alumni Research Foundation for generous grants in support of this work.

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RECEIVED DECEMBER 24, 1958

THE USE OF HIGH EFFICIENCY CAPILLARY COLUMNS FOR THE SEPARATION OF CERTAIN *cis-trans* ISOMERS OF LONG CHAIN FATTY ACID ESTERS BY GAS CHROMATOGRAPHY[1]

Sir:

A new concept in gas chromatography was introduced by Golay,[2] who suggested the use of columns made by coating the inner surface of narrow bore capillary tubing with a thin layer of stationary phase. Such columns possess a performance and operating efficiency far greater than is possible with conventional packed columns. The very small quantity of stationary phase lining the inner surface of the capillary tube requires, however, sample loads in the region of one microgram or less if the performance of the column is to be realized in full. This in turn makes severe demands on the detector used to sense the low vapor concentrations emerging from the column. An ionization detector with a sensitivity of 10^{-13} mole and a sensing volume of only a few microliters was described recently by Lovelock.[3] This detector, modified, formed part of the apparatus used in this investigation.

Table I shows the results of a gas chromatographic analysis of a known mixture of the methyl esters of saturated and unsaturated fatty acids extending from C-8 to C-20. A 200 foot stainless steel capillary column with an internal diameter of 0.010 inch which was coated with Apiezon "L" was used. The column was maintained at 240°. The inlet pressure of the argon carrier gas was 0.68 atm.; the outlet flow rate was 0.5 ml./min. The sample was introduced into the column by means of a T-shaped glass bypass device maintained at 300°. In this manner approximately 99.9% of the volatilized sample was vented to the atmosphere. The remainder, approximately one gamma, entered into the capillary column.

TABLE I

Methyl ester	Composition, %	Corrected retention time, min.	S_f[a]	Calcd. theor. plates
Octanoate	4.1	2.8	0.04	21,400
Nonanoate	6.8	4.4	.07	25,400
Decanoate	3.5	6.4	.10	30,600
Undecanoate	1.2	9.6	.15	40,000
Laurate	9.0	14.1	.21	55,400
Tridecanoate	2.9	20.6	.31	60,200
Myristate	10.7	30.6	.47	64,100
Pentadecanoate	0.2	44.4	.68	80,800
Palmitoleate	1.4	58.6	.89	101,800
Palmitate	14.2	65.6	1.00	36,800
Margarate	4.4	95.1	1.45	94,500
Linolenate	7.5	118	1.80	...
Linoleate	3.7			
Oleate	14.9	124	1.89	31,800
Elaidate	2.2	125	1.91	76,500
Stearate	8.4	139	2.12	59,200
Arachidonate	1.7	204	3.11	200,000
Arachidate	3.2	296	4.52	128,000

[a] Separation factor based on methyl palmitate equal to 1.00.

Under these experimental conditions an extremely efficient column was obtained making possible for the first time the separation of certain *cis–trans* isomers, *i.e.*, methyl elaidate from methyl oleate (Table I).

The highest calculated theoretical plate efficiency for any one component was 200,000 (methyl arachidonate) or 1,000 plates per foot. Despite the fact that the Apiezon coated capillaries provided excellent efficiencies, the separation of methyl linoleate from methyl linolenate was not achieved.

Preliminary experiments employing capillary columns containing certain polyesters as stationary liquids[4] provided the rapid resolution of most components including linoleate and linolenate with good separation factors but low theoretical plate efficiencies.

(1) This work was supported by the National Heart Institute of the National Institutes of Health, the National Dairy Association and the Nutrition Foundation.

(2) M. J. E. Golay, "Gas Chromatography," Academic Press, Inc., New York, N. Y., 1958.

(3) J. E. Lovelock, *Nature*, **182**, 1663 (1958).

(4) S. R. Lipsky, R. A. Landowne and M. R. Godet, *Biochim. Biophys. Acta*, **31**, 336 (1959).

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RECEIVED DECEMBER 18, 1958

SYNTHESIS OF 18-OXYGENATED PROGESTERONES

Sir:

Steroid metabolites, oxygenated at C-18 but lacking an oxygen at C-11, have been detected recently.[1,2] However, biological evaluation of this new type of compounds has been hampered because of the minute amounts available. We wish to report therefore a practical method for the conversion of the readily available alkaloid conessine (I) to C-18 oxygenated progesterones and other related steroids.

Conessine (3β-dimethylamino-con-5-enine,[3] I) on treatment with sodium borohydride and aluminum

(1) K. H. Loke, G. F. Marrian, W. S. Johnson, W. L. Meyer and D. D. Cameron, *Biochim. Biophys. Acta*, **28**, 214 (1958).

(2) R. Neher and A. Wettstein, *Helv. Chim. Acta*, **39**, 2062 (1956).

(3) R. D. Haworth and M. Michael, *J. Chem. Soc.* 4973 (1957).