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., $[\alpha]^{23}D$ −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).

DEPARTMENT OF MEDICINE S. R. LIPSKY
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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).

chloride in diglyme,[4] followed by chromic acid oxidation of the resulting 6-boron conanine derivative (II), gave 3β-dimethylaminoconanin-6-one (III), m.p. 198–203°,[5] $[\alpha]^{25}D$ +11.1°, λ_{max}^{KBr} 5.88 μ, C, 77.46; H, 10.59; N, 7.80, dinitrophenylhydrazone dec. about 250°. Reaction of the corresponding bismethiodide (IV) with potassium *t*-butoxide in boiling *t*-butyl alcohol afforded 18-dimethylamino-3,5-cyclopregn-20-en-6-one (V), m.p. 73–75° and 87–88°, $[\alpha]^{25}D$ +51.0°, λ_{max}^{KBr} 5.93 and 6.12 μ, C, 80.82; H, 10.30, in over 80% yield.

The metho-*p*-toluenesulfonate of V (VI) was hydroxylated with aqueous potassium chlorate and osmium tetroxide as a catalyst to yield a mixture of the corresponding 20α,21-diol (VIIa) and 20β,21-diol (VIIb), which was then refluxed with potassium *t*-butoxide in *t*-butyl alcohol, affording 21-hydroxy-18,20β-epoxy-3,5-cyclopregnan-6-one (VIII), m.p. 185–188°, $[\alpha]^{25}D$ +62.9°, λ_{max}^{KBr} 2.84 and 5.95 μ, C, 76.36; H, 9.13, as the major neutral product.

Treatment of VIII with *p*-toluenesulfonyl chloride in pyridine resulted in the corresponding tosylate IX, m.p. 142–143°, which on reaction with dimethylamine yielded the 21-dimethylamino derivative (X), m.p. 145–147°, C, 77.20; H, 10.05; N, 4.20. Reduction of X with lithium aluminum hydride led to a mixture of epimeric 6-hydroxy derivatives, m.p. 133–137°, that was isomerized with formic acid to the corresponding 21-dimethylamino-18,20β-epoxy-5-pregnen-3-ol (XI), m.p. 160–163°. Oppenauer oxidation of XI yielded 21-dimethylamino-18,20β-epoxy-4-pregnen-3-one (XII), m.p. 123–124.5°; λ_{max}^{KBr} 5.98, 6.20 μ and 240.5 mμ, ε 17,140. The corresponding N-oxide (XIII) smoothly eliminated dimethylhydroxylamine in refluxing *t*-butylbenzene, affording 18,20-epoxy-4,20-pregnadien-3-one (XIV), $\lambda_{max}^{CS_2}$ 5.95, 6.19 and 12.57 μ.

The enol ether XIV in presence of dilute acids, was hydrolyzed readily to 20-hydroxy-18,20-epoxy-4-pregnen-3-one (XV), m.p. 173–182°, λ_{max}^{KBr} 2.92, 5.96, 6.18 μ and 241 mμ, ε 17,100, C, 76.56; H, 9.17, which was shown to be, by paper chromatography, a mixture of readily interconvertible 20α and 20β alcohols, that had little tendency to react in the tautomeric 18-hydroxyprogesterone form.[6] However, oxidation of XV with chromic acid in pyridine gave 3,20-diketo-4-pregnen-18-oic acid (XVI), m.p. 225–227°, λ_{max}^{KBr} 2.94, 5.70, 6.00, 6.20 μ and 240.2 mμ, ε 17,200, C. 73.07; H, 7.98, existing as the tautomeric hydroxy-lactone; the neutral fraction contained, besides much starting material, 3,20-diketo-4-pregnen-18-al (XVII) m.p. 139–142°, λ_{max}^{KBr} 3.67, 5.83, 6.00, 6.22 μ and 240.4 mμ, ε 17,000.

In order to establish the structure of the enol ether (XIV), the product was treated with osmium tetroxide, yielding 20,21-dihydroxy-18,20-epoxy-4-pregnen-3-one (XVIII),[6,7] m.p. 191–195°, λ_{max}^{KBr} 2.90, 6.00 and 6.20 μ, C, 72.72; H, 8.75, which reacted with lead tetraacetate, affording 18-hydroxy-3-keto-4-etien-20-oic acid 20,18-lactone (XIX), m.p. 227–231°, λ_{max}^{KBr} 5.62, 5.99 and 6.19 μ (lit.[7] m.p. 221–224°). This product was identical with a lactone isolated in these laboratories during experiments involving the perfusion of desoxycorticosterone through beef adrenals.[8]

(4) H. C. Brown and B. C. Subba Rao, THIS JOURNAL, **78**, 5694 (1956).

(5) Rotations were determined in commercial acid-free chloroform.

(6) Acetylatiou of XVIII with acetic anhydride in pyridine overnight—conditions which did not essentially affect XV—gave a monoacetate, m.p., 158–159°, λ_{max}^{Kbr} 2.90, 5.77 5.99 and 6.22 μ.

(7) *Cf.* F. W. Kahnt, R. Neher and A. Wettstein, *Helv. Chim. Acta*, **38**, 1237 (1955), who claimed to have isolated 18,21-dihydroxy-4-pregnen-3,20-dione from a mixture obtained by incubation of desoxycorticosterone with adrenal homogenates.

(8) Dr. J. S. Mihina, private communication.

DIVISION OF CHEMICAL RESEARCH
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RECEIVED DECEMBER 22, 1958

AN ENZYMATIC CHLORINATION REACTION

Sir:

Despite the frequent occurrence of chlorine-containing natural products, the biological mechanism for the formation of the carbon–chlorine bond is unknown. We wish to report the synthesis of the carbon–chlorine bond catalyzed by an enzyme (or enzyme system) from *Caldariomyces fumago*. The major chlorine-containing metabolite excreted by this organism is caldariomycin, which has been identified tentatively as 2,2-dichloro-1,3-cyclopentanediol[1].

Acetone-dried mycelial powders of this mold catalyze the conversion of chloride ion (Cl^{36}) into an ether extractable organic form when supplemented with β-ketoadipic acid (Table I). The requirement for β-ketoadipic acid is specific; a number of related β-keto carboxylic acids as well as other common metabolic intermediates have been tested and do not show significant activity. Table I also illustrates the enzymatic nature of this reaction since heat denatured mycelial powders do not catalyze the reaction.

TABLE I

THE ENZYMATIC CONVERSION OF CHLORIDE ION TO AN ETHER EXTRACTABLE ORGANIC FORM

The complete system contained 500 μmoles of potassium phosphate buffer, *p*H 6.0; 10 μmoles of potassium Cl^{36} (specific activity of 8900 c.p.m./μmole); 100 μmoles of potassium β-ketoadipate and 100 mg. of acetone-dried mycelial powder in a total volume of 5 ml. Following 1 hour of aerobic incubation at 25°, the reaction mixture was acidified to *p*H 3 with 7*N* sulfuric acid and the aqueous phase extracted twice with 2 volumes of diethyl ether. The ether extract was dried over sodium sulfate, concentrated on a steam-bath under nitrogen, plated and counted in a gas flow counter.

	Additions	Cl^{36} Incorporation c.p.m.	μmoles
1	Complete	890	0.1
2	1 minus β-ketoadipate	30	0.003
3	1 minus Cl^{36}	0	0
4	1 with heat denatured mycelial powder	0	0

Large scale incubations allowed the accumulation of approximately 5 mg. of the radioactive, enzymatically synthesized compound. The behavior of this unknown compound on a Dowex-1 column (formate phase) indicated it to be a weaker acid than β-ketoadipic acid. The organically

(1) P. W. Clutterbuck, S. L. Mukhopadhyay, A. E. Oxford, and H. Raistrick, *Biochem. J.*, **34**, 664–677 (1940).