in better than 98% purity by the dehydration of 3,3-dimethyl-1-butanol over this catalyst. Previous methods of dehydrating this alcohol were accompanied by appreciable rearrangement.[5]

The mechanism suggested here seems to be the most reasonable. However, one involving participation by a neighboring group is not excluded.[6]

Further work with these and other alcohols and with bases other than ammonia and the study of the details of the mechanism are currently being carried out.

(5) V. N. Ipatieff, W. W. Thompson and H. Pines, THIS JOURNAL, **73**, 553 (1951).

(6) S. Winstein, B. K. Morse, E. Grunwald, K. C. Schreiber and J. Corse, *ibid.*, **74**, 1113 (1952), and other papers in that series.

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RECEIVED MARCH 14, 1960

THE BIOSYNTHESIS OF TETRACYCLINE ANTIBIOTICS

Sir:

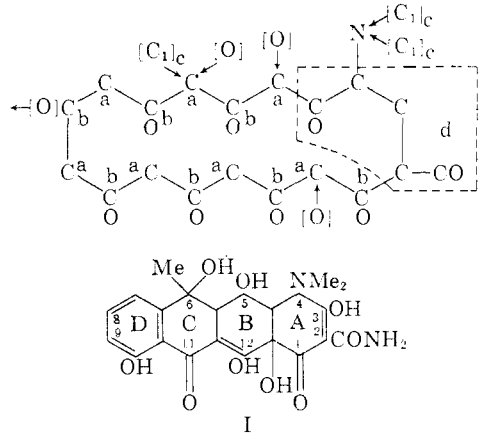
Inspection of the formulas of tetracycline antibiotics, *e.g.*, oxytetracycline, I, suggests that a considerable part of the molecule may arise biosynthetically by the acetic acid route,[1,2,3] with the probable introduction of one methyl group bonded to carbon and two methyl groups bonded to the amino nitrogen group.

The first experimental support for this idea was provided by Snell, *et al.*,[2] who showed that sodium acetate-2-C^{14} was a fermentation precursor (incorporation 5–10%) by a fairly direct route, since the specific activity of the product was directly proportional to the quantity of tracer added. Degradation[2] showed less activity in ring A than in rings B, C and D. Glutamate has been found to be a unique amino-acid source of oxytetracycline in a semi-synthetic medium, and addition to a fermentation of (±)glutamic acid-2-C^{14} gave an active oxytetracycline. Degradation of the product showed that terracinoic acid (from rings B, C, D) contained only 5% of the activity, suggesting that glutamate may be a fairly direct precursor of part at least of ring A.[3]

Detailed degradations have now been carried out on oxytetracycline derived from (i) $C^{14}H_3$-methionine and (ii) 2-$C^{14}H_3CO_2H$. (i) Oxytetracycline (r.m.a.[4] 50.9×10^3) gave by Kuhn–Roth oxidation CH_3CO_2H (r.m.a. of $BaCO_3$ from the CH_3 group, 17.3×10^3; r.m.a. of $BaCO_3$ from the CO_2H, 0) and by standard degradation[5] to tetramethylammonium iodide (r.m.a. 34.8×10^3). All the activity, as expected, is in the C_6-CH_3 and the $N(CH_3)_2$, the activity of all CH_3 groups being the same. (ii) Oxytetracycline (r.m.a. 42.2×10^3) from $C^{14}H_3CO_2H$ after degradations[5] gave products with these r.m.a. $\times 10^{-3}$: terracinoic acid, 27.1; 7-acetoxy-3-methylphthalide 18.7; decarboxyterracinoic acid 26.4; $BaCO_3$ from C_{11}, 0.3; $BaCO_3$ from C_6, 4.0; $BaCO_3$ from C_6-CH_2, 0.3; $BaCO_3$ from C_2-$CONH_2$, 0.6; tetramethylammonium iodide, 0.5.

These results are quantitatively in accord with the production of the molecule by the head to tail linkage of acetic acid units at least from C_5-C_{12} (except C_6-CH_3). It is necessary to assume that the incorporated unit from $C^{14}H_3CO_2H$ has its activity randomized to the extent of 5% and that the methyl-pool has become slightly labelled from this source. It is likely that the glutamate portion extends from the C_2-$CONH_2$, which is differently labelled to an acetate carboxyl, through C_2 to C_{4a}. Degradations to deal with this part of the molecule are inefficient.

The results are in accord with the distributions of label shown in I: (i) r.m.a. contributions of a, b, d = 0, c = 17.3×10^3; (ii) r.m.a. contributions of a = 4×10^3; b = 0.3×10^3; c = 0.25×10^3; with glutamic acid-2-C^{14} as source the r.m.a. contribution of d is much greater than a, b and c.



I

We are indebted to Dr. Herchel Smith for assistance with some of the tracer measurements; financial aid from the Rockefeller Foundation and Chas. Pfizer & Co., Inc., supported parts of the work.

(6) A. J. Birch, R. A. Massy-Westropp, R. W. Rickards and H. Smith, *J. Chem. Soc.* (*London*), 360 (1958).

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RECEIVED MARCH 21, 1960

A NEW CLASS OF ACTIVE STEROIDS: THE 19-NOR-$\Delta^{4,9}$-3-KETOSTEROIDS

Sir:

Since the initial demonstration that Δ^1-unsaturation of the corticoids results in enhanced biological and therapeutic activity,[1] numerous investigations have been made of other conjugated systems. The $\Delta^{4,6}$-unsaturated derivatives of the

(1) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science*, **121**, 176 (1955).

(1) A. J. Birch, Fortschr. Chem. Org. Naturstoffe, Springer, Vienna, **14**, 186 (1957); R. Robinson, "Structural Relations of Natural Products," Oxford Press, New York, N. Y., 1955.

(2) J. F. Snell, R. L. Wagner and F. A. Hochstein, "Internat. Conf. on Peaceful Uses of Atomic Energy," **12**, 431 (1956).

(3) J. F. Snell, in "Radioactivity for Pharmaceutical and Allied Research Laboratories," Academic Press, Inc., New York, N. Y., in press.

(4) Relative molar activity, proportional to molar activity *cf.*[6]

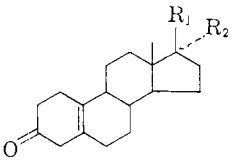
(5) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternak, P. N. Gordon, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **75**, 5455 (1953), and references cited therein.

steroid hormones in the 19-methyl-[2] and 19-nor-[3] series have been evaluated, and the $\Delta^{1,4,6}$-system[4] also has been reported. We now wish to report the ready synthesis of yet another biologically active class of unsaturated analogs, the 19-nor-$\Delta^{4,9}$-3-ketosteroids (II).[5,6]

This unusual, stable conjugated system results directly from one-step bromination of $\Delta^{5(10)}$-3-ketosteroids (I) in pyridine solution. The pyridine solvent is unique since the intermediate 5,10-dibromo- or 10-bromo-Δ^4-3-ketosteroids[7] in solutions of acetic acid, chloroform, etc., or even in the solid state, decompose to yield predominantly phenolic products.

Treatment of 19-nor-$\Delta^{5,(10)}$-androsten-17β-ol-3-one (Ia)[8] with one equivalent of bromine, or, more conveniently, with pyridine perbromide hydrobromide by this new process gives 19-nor-$\Delta^{4,9}$-androstadien-17β-ol-3-one (IIa), m.p. 187–188°, $[\alpha]_D$ −290° (acetate, m.p. 107°, $[\alpha]_D$ −237°). Chromic acid oxidation of IIa yields 19-nor-$\Delta^{4,9}$-androstadiene - 3,17 - dione (IIb), m.p. 130–131°, which is also obtained directly by bromination (pyridine) of 19-nor-$\Delta^{5(10)}$-androstene-3,17-dione (Ib). Similar brominations of Ic (m.p. 132–134°), Id[9] and Ie[10] give the correspondingly substituted 19-nor-$\Delta^{4,9}$-androstadien-17β-ol-3-ones, IIc (17α-methyl), polymorphic, highest m.p. 143–145°; IId (17α-ethyl), m.p. 77–79°; IIe (17α-ethinyl), m.p. 154–156°, $[\alpha]_D$ −322°.

Although selective bromination of the 5(10)-double bond is achieved easily when the 17α-substituent is acetylenic (Ie), such selectivity is not possible with the 17α-vinyl compound (If). Consequently, two equivalents of bromine are used, and the resulting crude 20,21-dibromo-$\Delta^{4,9}$-3-keto-steroid is treated with sodium iodide–acetone to give 17α-vinyl-19-nor-$\Delta^{4,9}$-androstadien-17β-ol-3-one (IIf), m.p. 136–140°. Preferably, IIf is obtained by selective hydrogenation of the ethinyl compound (IIe)(Pd/$BaSO_4$–pyridine). Ig is also converted to the $\Delta^{4,9}$-analog and treated with chromic oxide to afford 19-nor-$\Delta^{4,9}$-pregnadiene-3,20-dione (IIh), which has resisted all attempts at crystallization.



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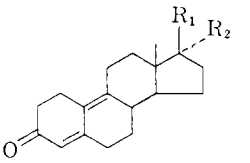

II

a, R_1 = OH, R_2 = H
b, $R_1 + R_2$ = O=
c, R_1 = OH, R_2 = CH_3
d, R_1 = OH, R_2 = C_2H_5
e, R_1 = OH, R_2 = C≡CH
f, R_1 = OH, R_2 = CH = CH_2
g, R_1 = CH(OH)CH_3, R_2 = H
h, R_1 = $COCH_3$, R_2 = H

Studies on the chemistry of the $\Delta^{4,9}$-bisdehydro system and on the chemical and microbiological preparation of additional analogs, particularly in the C_{20} series, are in progress and will be reported shortly.

In general, the biological activity[11] of the $\Delta^{4,9}$-series resembles the 19-nor-Δ^4-compounds. However, there are some marked differences in that IIc, IId and IIf are exceptionally potent oral antiestrogens and IIe is more effective than 17α-ethinyl-19-nortestosterone in reducing fecundity in rats.

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RECEIVED MARCH 17, 1960

(2) V. R. Mattox, E. L. Woroch, G. A. Fleisher and E. C. Kendall, *J. Biol. Chem.*, **197**, 261 (1952).

(3) F. B. Colton, U. S. Patent 2,874,170 (1959).

(4) D. Gould, E. L. Shapiro, H. L. Herzog, M. J. Gentles, E. B. Hershberg, W. Charney, M. Gilmore, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, THIS JOURNAL, **79**, 502 (1957); E. J. Agnello and G. D. Laubach, *ibid.*, **79**, 1257 (1957).

(5) This structure has been suggested for a component of the crude oil obtained by dehydration of 19-nor-Δ^4-androsten-11β-ol-3,17-dione: A. Zaffaroni, H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas and C. Djerassi, *ibid.*, **80**, 6110 (1958).

(6) Correct analyses have been obtained for all new compounds described with the exception of the non-crystalline material IIh for which verifying spectral data have been obtained. The $\Delta^{4,9}$-3-one system absorbs at 302–4 mμ, ϵ = 18,000–21,000 (ethanol) and at 6.04 ±0.01 μ (carbonyl), 6.22 ± 0.01 μ (diene) and 6.29 ± 0.02 μ (diene shoulder) (chloroform).

(7) Interruption of the reaction at a suitably short interval after bromine addition allowed the isolation of these intermediates.

(8) A. L. Wilds and N. A. Nelson, THIS JOURNAL, **75**, 5366 (1953).

(9) F. B. Colton, L. N. Nysted, B. Riegel and A. L. Raymond, *ibid.*, **79**, 1123 (1957).

(10) F. B. Colton, U. S. Patent 2,725,389 (1955).

(11) Endocrinological testing was carried out in the Lilly Laboratories (R. J. K.) and will be reported in more detail elsewhere.