a quite significant amount of radioactivity appeared in the nicotine.

TABLE I

	Compound supplied	Acid specific activity (d.p.m./mg. C or H)	Nicotine yield, mg.	Nicotine specific activity (d.p.m./mg. C or H)
1	75 mg. nicotinic acid-H^3	6.4×10^6	25	1.73×10^6
2	44 mg. nicotinic acid-H^3	6.4×10^6	25	1.33×10^6
3	9 mg. nicotinic acid-C^{14}	1910	24	102

Oxidation of the tritium-labeled nicotine (sample 1, Table I) with hot concentrated nitric acid yielded nicotinic acid having a specific activity of 2.97×10^6 d.p.m./mg. H. Owing to the possibility that isotope exchange might have occurred under such drastic conditions, the oxidation was repeated with aqueous potassium permanganate as reagent. Nicotinic acid was obtained which had a specific activity of 4.74×10^6 d.p.m./mg. H. This can be compared to the activity of the nicotine (sample 1, Table I), which is 4.84×10^6 d.p.m./mg. H *as nicotinic acid* (assuming the pyrrolidine hydrogens to be inactive), and thus represents an almost complete recovery of tritium from the nicotine.

Further details of these and other supporting experiments will appear in a later paper.

It is thus probable that the pyridine ring of nicotinic acid is a biosynthetic precursor of the pyridine ring of nicotine and of nornicotine[12] and perhaps also of the related tobacco alkaloids, myosmine, anabasine, anatabine, etc. It is of interest to note that this work, together with that of Dewey, Byerrum and Ball[8] and Leete,[9] constitutes the first recorded biosynthetic pathway for a plant alkaloid. It is further noteworthy that a universally distributed cofactor of biological catalysts and an important amino acid metabolite should be involved as intermediates in the syntheses of one of a class of substances for which no biochemical or physiological significance has yet been adduced.

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AN ASYMMETRIC COPOLYMER SYNTHESIS

Sir:

The multiple possibilities for stereoisomerism in vinyl polymers containing monosubstituted mers was early used by Staudinger[1] to explain their generally amorphous character and by Huggins[2] to explain the dependence of their physical properties on temperature of polymerization.[3]

Practical methods have now been devised to prepare more crystalline monosubstituted vinyl polymers, which are said to contain an alternating sequence of D and L configurations[4] and others apparently containing sequences of adjacent mers of like configuration.[5,6]

The closely related problem of preparing a polymer or copolymer with an excess of one configuration in the backbone of the polymer chain and exhibiting optical activity was first attacked unsuccessfully about sixty years ago.[7] Since then further attempts have been made by initiating polymerization with optically active acyl peroxides[8] and by polymerizing and copolymerizing optically active monomers.[9,10] Although the latter method might be expected to induce an excess of one configuration during polymerization, removal of the optically active centers initially present has so far left inactive polymers.

In the following synthesis we have found evidence of induced asymmetry during vinyl polymerization: 1-α-methylbenzyl alcohol (I) ($(M)^{25}\text{D}$ $-41.5°$, $a = -17.0°$, $l = 0.5$ cm.)[11] was allowed to react with methacrylyl chloride[12] in pyridine. *l*-α-Methylbenzyl methacrylate (II) (b.p. 92° at 3–4 mm.) was isolated by conventional methods in 65% yield. (*Anal.* Calculated for $C_{12}H_{14}O_2$: C, 75.78; H; 7.4. Found: C, 75.8; H, 7.7. $[M]^{25}\text{D}$ $-78.8°$, $a = -20.74°$; $l = 0.5$ dcm.).

(II) was polymerized in the absence of air in peroxide-free dioxane at 35° for 32 hours or more. α,α'-Azobisisobutyronitrile (ABIN) photosensitized by irradiation from a Type AH4 Hanovia Mercury lamp (General Electric Co.) was used as initiator.[13] The polymer (III) isolated by precipitations in petroleum ether and freeze drying in dioxane was found to have an ultraviolet absorption spectrum with peaks at 252, 258 and 264 mμ, characteristic of the α-methylbenzyl group, and a negative rotation. ($[M]^{25}\text{D}$ -147, $a = -0.87$, $l = 0.5$ dcm., $c = 2.2\%$ in dioxane.) Calcd. for $(C_{12}H_{14}O_2)_n$: C, 75.7; H, 7.4. Found: C, 75.2; H, 7.7.

The α-methylbenzyl groups were removed by heating 0.300 g. of (III) with 2 g. of phosphonium iodide (PH_4I) in acetic acid at 60°.[14]

The peaks of the ultraviolet absorption spectrum of the reduced polymer disappeared into the background absorption on reduction; the analysis indicated complete removal of the ester groups (calcd. for $(C_4H_6O_2)_n$: C, 55.9; H, 7.0. Found: C, 55.7; H, 7.5) and as expected[15] the resulting polymer (IV) showed no optical activity when observed under the same conditions as above.

When (II) was similarly copolymerized with maleic anhydride in a 1:3 molar ratio of ester to maleic

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anhydride, a copolymer (V) was formed with an absorption spectrum containing the same peaks and an analysis indicating a ratio of 1.5 methacrylate units to one maleic anhydride. (Calculated for $(C_{22}H_{23}O_6)_n$: C, 69.0; H, 6.01. Found: C, 68.94; H, 6.13.) The molar rotation per benzyl unit was less than that of the polymer, although little difference would be expected if the rotation of the copolymer was due solely to the methylbenzyl groups[16]: ($[M]^{25}D$ −128°, $a = -0.53°$, $l = 0.5$ dcm., $c = 2.1\%$ in dioxane).

When the original asymmetric centers were removed by phosphonium iodide, the reduced copolymer (VI), exhibited no characteristic absorption peaks at the previously mentioned wave lengths and it showed optical rotation of the opposite sign: ($[M]^{25}D$ +33.2, $a = +0.22$, $l = 0.5$ dcm., $c = 2.2\%$ in dioxane).

Although the analysis suggests more reduction than expected (calcd. for $(C_{10}H_{11}O_6)_n$: C, 52.9; H, 4.85. Found: C, 54.32; H, 7.24), the only explanation for the observed rotations of the reduced and unreduced copolymer appears to be that asymmetry has been induced during the process of radical polymerization.

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[16] "Optical Activity and Chemical Composition," by H. Landolt, translated by J. McCrae, Whittaker & Co., London, 1899, pp. 131, 132.

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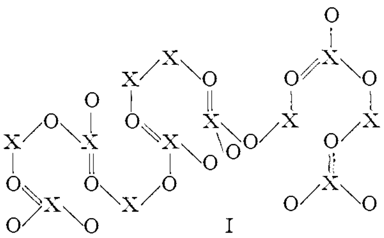
THE BIOSYNTHESIS OF STEROIDS AND TRITERPENES. THE ORIGIN OF CARBONS 11 AND 12 OF ERGOSTEROL[1]

Sir:

The squalene hypothesis[2] for the mechanism of biosynthesis of steroids and triterpenes has been investigated widely and all the evidence obtained has substantiated the concept.[3] Since the central two carbon atoms of squalene are derived from the carboxyl of acetate,[4] this symmetry presents a demanding requirement for the concept of the utilization of intact squalene (I) in the biosynthesis of steroids. These two central carbon atoms of squalene must be in the juxtapositions, C_{11} and C_{12}, of ring C of the steroids. Degradation of ring C of such a compound would yield information with regard to this unique symmetry requirement. The sterol chosen to study was ergosterol (II) since the presence of a homoannular diene in ring B facilitates the preparation of compounds required for the degradation of ring C.



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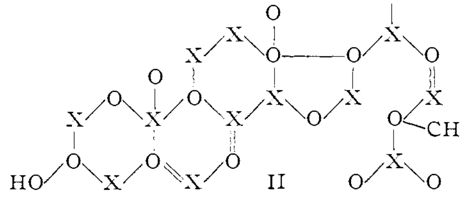

II

C^{14}-ergosterol, obtained from *Saccharomyces cerevisiae* grown in the presence of carboxyl-labeled acetate, was converted to 3β-acetoxy-11-ketoergostane (III)[5-8] and C_{11} and C_{12} obtained individually from III by the following series of reactions. For the degradation leading to C_{11}, III was converted to 3β,11-diacetoxyergost-9(11)-ene (IV)[9] which, in turn, was ozonized and saponified to yield 3β-hydroxy-9-keto-9,11-secoergostane-11-oic acid (V) [m.p. 172.5–173.5°; $[\alpha]^{25}D$ −57° Chf.; C, 74.82; H, 10.75; neut. equiv., 449]. The acid V was allowed to react with HN_3 and C_{11} was obtained as CO_2.

For the degradation leading to C_{12}, III first was reduced to the 11β-ol (VI)[9] which was dehydrated to yield 3β-acetoxyergost-9(11)-ene (VII).[6] VII was oxidized with *t*-butyl chromate and the 3β-acetoxy-12-ketoergost-9(11)-ene (VIII) [m.p. 133.5–134.5°; $[\alpha]^{25}D$ +45° Chf.; λ_{max}^{EtOH} 238 mμ (ε 10,900); C, 79.12; H, 10.62] then was hydrogenated over Pd–$CaCO_3$ to form 3β-acetoxy-12-ketoergostane (IX) [m.p. 174–175°; $[\alpha]^{25}D$ +53 Chf.; C, 78.31; H, 10.83]. IX was allowed to react with perbenzoic acid[10] and the 3β-acetoxy-13-hydroxy-12,13-secoergostane-12-oic acid 12,13 lactone (X) [m.p. 158–159°; $[\alpha]^{25}D$ −14° Chf.; C, 75.83; H, 10.33] formed was saponified in aqueous methanolic KOH to yield 3β,13-dihydroxy-12,13-secoergostane-12-oic acid (XI) [m.p. 188.5–189.3°; $[\alpha]^{25}D$ 0° MeOH; C, 74.36; H, 11.18; N.E. 431]. The acid XI was allowed to react with HN_3 and C_{12} obtained as CO_2.

It was found that both C_{11} and C_{12} were derived from the carboxyl of acetate and were equally labeled (C_{11}, 13.5 dis./min./mg. $BaCO_3$; C_{12}, 12.8 dis./min./mg. $BaCO_3$). The values found for the specific activities of these carbon atoms were less than that calculated (17.5 dis./min./mg. $BaCO_3$) on the basis of the squalene hypothesis. However, this could well be due to the large counting error (20%)

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[1] This work was supported, in part, by Grant No. AT (11-1), Project No. 16, U. S. Atomic Energy Commission.

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