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.

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

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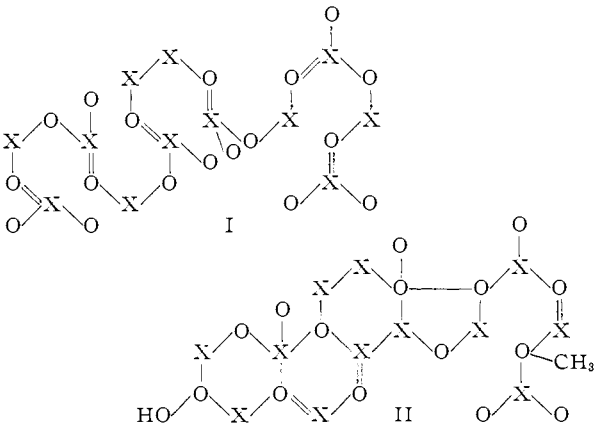
THE BIOSYNTHESIS OF STEROIDS AND TRITERPENES. THE ORIGIN OF CARBONS 11 AND 12 OF ERGOSTEROL[1]

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.



I

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%)

(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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(9) A. Crawshaw, H. B. Henbest and E. R. H. Jones, *ibid.*, 731 (1954).

(10) E. S. Rothman, M. E. Wall and C. R. Eddy, THIS JOURNAL, **76**, 527 (1954).

resulting from the very low activity of the ergosterol (7.5 dis./min./mg. $BaCO_3$).

It can be concluded that steroids derived from carboxyl-labeled acetate are labeled in the juxtapositions, C_{11} and C_{12}, as demanded by the squalene hypothesis and such a result strongly supports a concept of the intact utilization of the acyclic triterpene, squalene.

We wish to thank Professor D. J. Hanahan of the University of Washington for kindly supplying the C^{14}-ergosterol, Merck and Co., Inc., for a generous gift of ergosterol derivatives, and Dr. E. M. Baker of the Radiation Laboratory, University of California, for the C^{14} determinations.

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INHIBITION OF REGENERATION IN HYDRA BY CERTAIN NEW 6-(PHENYLALKYL)-AMINOPURINES

Sir:

Methods have been developed for quantitatively studying the processes of regeneration in hydra, a primitive organism that may well serve as a model system of development and cell differentiation in higher animals.[1] Adenine and various adenine derivatives have been found to retard the formation of new tentacles in hydra whose hypostome and tentacles have been cut away. In an attempt to further characterize the nature of the effect, a variety of 6-(substituted)-purines have been synthesized and tested. Most of the compounds are considerably more active than adenine.

One series in particular, the 6-(ω-phenylalkyl)-aminopurines, is extremely active, especially certain higher homologs (Table I). In this animal system all members of the series are more effective than the recently reported cell division factor, for plants, kinetin (6-(2-furfuryl)-aminopurine),[2] which has an activity only 20 times that of adenine. The new compounds were prepared by condensing 3 to 5 parts of the appropriate amine[3] with one part of 6-methylmercaptopurine in a sealed micro Carius tube heated to 130 to 140° for 12 to 18 hours.[4] Excess solvent was removed under reduced pressure and the crystalline residue washed with cold alcohol and recrystallized from alcohol–water.

TALBE I

SYNTHESIS AND ACTIVITY OF 6-(ω-PHENYLALKYL)-AMINOPURINES

Compound 6-(R)-aminopurine	Yield, %	M.p., °C. (dec.)	Minimum concn. for full inhibition (μ mole/ml.)	Activity in terms of adenine
H-(Adenine)	..	...	5.0	1
1-Phenylmethyl-[a]	..	...	0.18	30
2-Phenylethyl-[b]	69	239–240	.04	130
3-Phenylpropyl-[c]	37	173–175	.02	250
4-Phenylbutyl-[d]	42	148–149	.003	1700
5-Phenylpentyl-[e]	40	145–147	.003	1700
7-Phenylheptyl-[f]	54	112–113	.001	5000

[a] C. G. Skinner and W. Shive, THIS JOURNAL, **77**, 6692 (1955). [b] *Anal.* Calcd. for $C_{13}H_{13}N_5$: C, 65.25; H, 5.47. Found: C, 65.14; H, 5.49. [c] *Anal.* Calcd. for $C_{14}H_{15}N_5$: C, 66.38; H, 5.97. Found: C, 66.19; H, 5.74. [d] *Anal.* Calcd. for $C_{15}H_{17}N_5$: C, 67.39; H, 6.41. Found: C, 67.13; H, 6.77. [e] *Anal.* Calcd. for $C_{16}H_{19}N_5$: C, 68.30; H, 6.81. Found: C, 68.19; H, 7.15. [f] *Anal.* Calcd. for $C_{18}H_{23}N_5$: C, 69.87; H, 7.49. Found: C, 69.99; H, 7.56.

Biological activity is expressed as the minimum concentration which will produce complete inhibition of visible tentacle formation after 18 hours at 27°. Relative activities are compared using adenine as a standard. All tests were conducted in a buffered (*p*H 7.4) solution containing all inorganic ions required for optimum rate of regeneration.

The strong inhibitions obtained at the very low concentrations of the higher analogs suggest that they block a fundamental controlling process rather than the gross metabolism of the organism. Current investigations are directed both at determining the structural specificity of the active compounds and at determining the system involved. A full report of the synthesis and testing of these and other 6-(substituted) purines is being submitted for publication.

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RECEIVED APRIL 2, 1956

(3) 3-Phenylpropylamine, 4-phenylbutylamine and 5-phenylpentylamine were prepared by catalytic hydrogenation of the nitriles using Raney nickel. 5-Phenylvaleronitrile was prepared from 5-phenylvaleric acid kindly furnished by Dr. P. D. Gardner. 7-Phenylheptylamine also was furnished by Dr. Gardner, unpublished data.

(4) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 412 (1952).

(5) National Science Foundation Predoctoral Fellow.

A REARRANGEMENT INVOLVING A 1,5-PHENYL MIGRATION

Sir:

We have observed that 8-benzhydryl-1-naphthoic acid (Ia) isomerizes under Friedel–Crafts conditions to a cyclic hemiketal (IIa). This reaction involves a 1,5-phenyl migration, and is the first example of a rearrangement of this type.

Very few, if any, acid catalyzed reactions have been described in which an alkyl or aryl group is transferred directly between carbon atoms that are not adjacently bound. A case that can be formulated conveniently as a 1,3-methyl migration has been reported[1]; however, the possibility that the product resulted from a sequence of conventional 1,2-migrations cannot be excluded. Recently, Meinwald[2] conclusively demonstrated that the isomerization of α-cinenic acid, a reaction for which a 1,5-methyl migration had been proposed, did not actually involve a methyl shift.

Compound Ia[3] (1.00 g.) was converted to the acid chloride with thionyl chloride, then warmed with 1.2 ml. of stannic chloride in 20 ml. of carbon disulfide for ninety minutes. Upon hydrolysis and recrystallization, 0.90 g. of IIa was obtained; m.p.

(1) R. G. Ham, D. C. Fitzgerald, Jr., and R. E. Eakin, *J. Exptl. Zool.*, manuscript submitted.

(2) C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza and F. M. Skoog, THIS JOURNAL, **77**, 2662 (1955).

(1) W. A. Mosher and J. C. Cox, THIS JOURNAL, **72**, 3701 (1950).

(2) J. Meinwald, *ibid.*, **77**, 1617 (1955).

(3) For the preparation of this compound see W. E. Bachmann and E. Chu, *ibid.*, **58**, 1118 (1936).