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.

CHEMICAL LABORATORY
UNIVERSITY OF CALIFORNIA — WILLIAM G. DAUBEN
BERKELEY 4, CALIFORNIA — THOMAS W. HUTTON

RECEIVED APRIL 9, 1956

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.

TABLE 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.

BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY — RICHARD G. HAM[5]
THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH — ROBERT E. EAKIN, CHARLES G. SKINNER
AUSTIN, TEXAS — WILLIAM SHIVE

RECEIVED APRIL 2, 1956

(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).

(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.

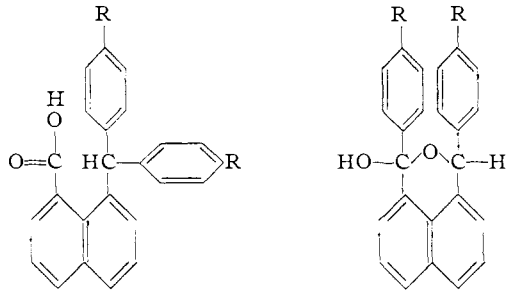
Very few, if any, acid catalyzed reactions have been described in which an alkyl or aryl group is transferred directly between carbon atoms that are not adjacently bound. A case that can be formulated conveniently as a 1,3-methyl migration has been reported[1]; however, the possibility that the product 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) 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).



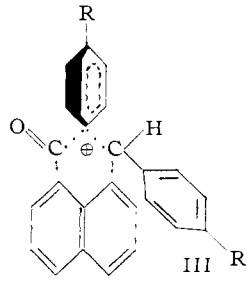
Ia, b IIa, b

a, R = H; b, R = OCH_3

137–137.5 (*Anal.* Calcd. for $C_{24}H_{18}O_2$: C, 85.18; H, 5.36. Found: C, 85.12; H, 5.21). The presence of hydroxyl and absence of carbonyl was indicated by the infrared spectra. IIa was also prepared (0.10 g.) by warming 0.50 g. of Ia with 16 ml. of sulfuric acid and 4 ml. of water for two hours on a steam-bath; 0.30 g. of Ia was recovered from this reaction.

Oxidation of IIa with chromium trioxide in acetic acid yielded (45%) 1,8-dibenzoylnaphthalene, m.p. and mixed m.p. 188.5–189.5°; reduction of IIa with lithium aluminum hydride in ether yielded (60%) a diol, m.p. 199–200 (*Anal.* Calcd. for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92: Found: C, 84.62; H, 5.88.), which was identical with the substance obtained by reducing 1,8-dibenzoylnaphthalene with lithium aluminum hydride. In further agreement with the assigned structure, IIa reacted with ethanol to form a ketal, m.p. 203–204.5, no hydroxyl or carbonyl by infrared (*Anal.* Calcd. for $C_{26}H_{22}O_2$: C, 85.21; H, 6.05. Found: C, 85.43; H, 6.00).

Further insight into the course of the reaction was provided by the rearrangement of the di-*p*-methoxy analog, Ib.[3] On successive treatment with thionyl chloride, stannic chloride and water, it was converted to IIb, m.p. 142–143 (*Anal.* Calcd. for $C_{26}H_{22}O_4$: C, 78.37; H, 5.57. Found: C, 77.79; H, 5.40). The structure of IIb is based on the analysis, infrared spectra, and the fact that oxidation with chromium trioxide yielded (47%) 1,8-di-*p*-anisoylnaphthalene (m.p. and mixed m.p. 217–218) as the only isolable product. This reaction shows that the migrating aryl group undergoes detachment and attachment at the same carbon atom.



III

We believe that the rearrangement is an intramolecular one, proceeding through a transition state of the type represented by III. The alternative intermolecular transfer of aryl groups seems highly improbable on steric grounds. Furthermore, the conditions of the reaction appear to be too mild to effect an intermolecular reaction.[4] This was demonstrated by refluxing a solution of benzoyl chloride, stannic chloride and triphenylmethane in carbon disulfide for fifteen hours. No ketonic material could be detected as a product and 92% of the triphenylmethane was recovered. Under similar conditions, I was isomerized in less than ninety minutes.

DEPARTMENT OF CHEMISTRY
NORTHWESTERN UNIVERSITY R. L. LETSINGER
EVANSTON, ILLINOIS P. T. LANSBURY[5]

RECEIVED APRIL 16, 1956

(4) Stannic chloride is a good reagent for closing rings by intramolecular acylation (W. S. Johnson, "Organic Reactions," Vol. II, p. 114), but it is not useful for the intermolecular acylation of benzene (G. Stadnikoff and A. Baryochewa, *Ber.*, **61**, 1996 (1928)).

(5) National Science Foundation Fellow, 1955–1956.

CHROMATOGRAPHY OF PROTEINS ON CELLULOSE ANION-EXCHANGERS USING WATER-CARBON DIOXIDE SYSTEMS

Sirs:

We have found that proteins can be chromatographed on cellulose anion-exchangers in a system of distilled water and carbon dioxide. This observation was unexpected since weak anion-exchangers do not normally remove carbon dioxide from water.[1] The proteins so fractionated were free of small ions. Recent publications have indicated that proteins can be fractionated on cellulose ion-exchangers.[2] However, these workers used a method involving buffer solutions of various ionic strengths making it necessary, in certain circumstances, to introduce additional steps for the removal of salts.

All the proteins tested, except the very basic ones, were sorbed from distilled water by the free-base form of the anion-exchanger. The proteins were not desorbed by simple washing with distilled water. In fact, usually the last traces of salt and some of the other non-protein materials were removed by the washing. The introduction of an atmosphere of carbon dioxide over the distilled water in contact with the protein-containing exchanger caused the release of part or all of the protein. Eluted proteins yielded solutions which, after the gas was permitted to escape, approached the isoionic *p*H's of the proteins. The exchanger can be regenerated with distilled-water washing, which removes carbon dioxide. In cases of accumulation of mineral acids, nucleic acids, and some proteins which are not removed by carbon dioxide, strong bases must be used to regenerate the exchanger.

Several proteins were examined batch-wise to help predict what to expect on a column. A protein, dissolved in distilled water or dialyzed against distilled water, was equilibrated for fifteen minutes with the 2-(diethylamino)-ethyl ether of cellulose (0.25–0.50 m.e./g.).[3] The sorbate was separated by centrifugation, washed, and eluted. Typical results are shown in Table I.

(1) R. Kunin and R. J. Myers, "Ion Exchange Resins," J. Wiley & Sons, New York, N. Y., 1950, p. 41.

(2) E. A. Peterson and H. A. Sober, THIS JOURNAL, **78**, 751, (1956); H. A. Sober, F. J. Gutter, M. M. Wyckoff and E. A. Peterson, *ibid.*, **78**, 756 (1956).

(3) C. L. Hoffpauir and J. D. Guthrie, *Textile Research J.*, **20**, 617 (1950).