creatic ribonuclease. In the latter case, uridine-2′,3′ cyclic phosphate was, as expected, an intermediate in the degradation. The synthetic material was, furthermore, chromatographically and electrophoretically identical with a sample of uridylyl-(5′→3′)-uridine prepared enzymically by the general method of Heppel, Whitfeld and Markham.[7]

Further work on the synthesis of C_5'–C_3' linked ribo-oligonucleotides is in progress.

(7) L. A. Heppel, P. R. Whitfeld and R. Markham, *Biochem. J.*, **60**, 8 (1955).

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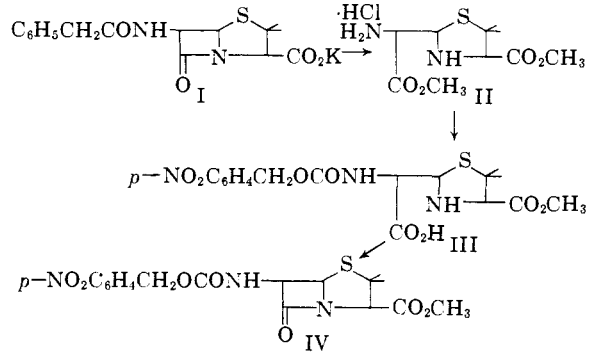
THE CHEMICAL CONVERSION OF PENICILLIN G INTO A BIOLOGICALLY ACTIVE SYNTHETIC PENICILLIN SERIES

Sir:

We wish to report the chemical removal of the phenylacetic acid side chain from penicillin G (I) to form compound II, thus opening up a promising route for the preparation of synthetic penicillins. Compound II has been converted into an intermediate (III) in a total synthetic series, thereby completing by relay a transition between a "natural" penicillin and a biologically active synthetic penicillin not directly available previously by fermentation.

Potassium benzylpenicillinate (penicillin G, potassium salt) was treated with methanol containing a catalytic amount of triethylamine to form potassium α-methyl D-α-benzylpenicilloate, which was converted directly in 22% over-all yield by reaction with methanolic hydrogen chloride to methyl D-α-4-carbomethoxy-5,5-dimethyl-α-amino-2-thiazolidineacetate hydrochloride (II), $C_{10}H_{19}ClN_2O_4S$, m.p. 174–175° dec., $\alpha^{25}D$ + 104° (C, 1.34 in methanol) [found: C, 40.28; H, 6.38; N, 9.34].

It was established that no change in configuration took place during the methanolysis by the conversion of II to the known[1] dimethyl D-α-benzylpenicilloate [m.p. 87–88°, $\alpha^{25}D$ + 82.2°] in 72% yield with phenylacetyl chloride and triethylamine. Identity with an authentic sample was established by comparison of optical rotation, melting point, mixed melting point and infrared spectra (KBr).



(1) H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, New Jersey, 1949, p. 613.

Acylation of the primary amine grouping in II was accomplished with *p*-nitrobenzyl chloroformate[2] and triethylamine to yield methyl-D-α-4-carbomethoxy-5,5-dimethyl-α-(carbo-*p*-nitrobenzyloxyamido)-thiazolidineacetate. Saponification of the α-methyl ester grouping with one equivalent of sodium hydroxide and crystallization from acetone–ether yielded D-α-4-carbomethoxy-5,5-dimethyl-α-(carbo-*p*-nitrobenzyloxyamido)-thiazolidineacetic acid (III), $C_{17}H_{21}N_3O_8S$; m.p. 138–139°, $\alpha^{27}D$ + 60.9° (C, 1.17 in methanol). [Found, C, 47.57; H, 4.98; N, 9.83.] The infrared spectrum (KBr) of this acid was identical to that of the corresponding DL-derivative prepared by total synthesis.[3] The infrared spectrum of the hydrochloride of III, m.p. 187–188°, [found, C, 44.39; H, 5.25; N, 8.84] was identical to that of the corresponding DL-hydrochloride when measured in dimethyl sulfoxide solution.

We are indebted to Bristol Laboratories of Syracuse, N. Y., for financial support and for bioassays.

(2) F. H. Carpenter and D. T. Gish, THIS JOURNAL, **74**, 3818 (1952).

(3) The DL form of this compound has been prepared in this laboratory by G. C. Stelakatos using the general procedure of J. C. Sheehan and P. A. Cruickshank (THIS JOURNAL, **78**, 3683 (1956)). DL-III has been cyclized to methyl DL-6-(carbo-*p*-nitrobenzyloxyamido)-penicillanate in 38% yield.

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THE CONFIGURATION OF MOLECULAR COMPLEXES

Sir:

Orgel and Mulliken[1] have suggested that molecular complexes may involve a variety of different relative orientations of the donor and acceptor. In order to determine the geometrical restrictions on charge-transfer interactions in molecular complexes, we synthesized and determined the spectra of a series of molecules having both the donor and acceptor groups in the same molecule and in a relatively fixed orientation with respect to each other. The donor in each case was the *p*-aminophenyl system and the acceptor, the *p*-nitrophenyl group. The compounds studied were 4-amino-4′-nitrodiphenylmethane (I), m.p. 96.9–97.5° (found for $C_{13}H_{12}O_2N_2$: C, 68.55; H, 5.21); 4-amino-4′-nitrobibenzyl (II), m.p. 136.8–137.5° (found for $C_{14}H_{14}O_2N_2$: C, 69.18; H, 5.56); 4-amino-4′-nitro-α,ω-diphenylpropane (III), m.p. 92.0–92.7°, (found for $C_{15}H_{16}O_2N_2$: C, 70.52; H, 6.40); *cis*-1-(4-aminophenyl)-2-(4-nitrophenyl)-cyclopentane (IV), m.p. 112.2–113.0° (found for $C_{17}H_{18}O_2N_2$: C, 72.25; H, 6.67); and *trans*-1-(4-aminophenyl)-2-(4-nitrophenyl)-cyclopentane (V), m.p. 76.5–77.3° (found for $C_{17}H_{18}O_2N_2$: C, 72.52; H, 6.53).

Compound I involves a 2.52 Å. separation for the 1-atoms of the ring and a 7.02 Å. separation for the 4-atoms. The aromatic rings in II and III can have an infinite variety of orientations with respect to each other because of rotation about the chain bonds. In IV the aromatic nuclei are practically

(1) L. E. Orgel and R. S. Mulliken, THIS JOURNAL, **79**, 4839 (1957).

face-to-face. In V the *trans* relation of the aromatic rings allows overlap of rings only at the 2-positions.

The electronic spectra of 1:1 methanol–water solutions of all these compounds were anomalous—the absorption intensities were greater than the sums of the intensities of the separate chromophores (approximated by *p*-toluidine and *p*-nitrotoluene). Absorption in the visible region resulted in definite coloration. The maxima in the anomalous absorption of these compounds are summarized in Table I.

TABLE I

ANOMALOUS ABSORPTION[a] OF 4-$NO_2C_6H_4$–$(CH_2)_x$–C_6H_4-NH_2-4′

Cpd.	x	λ'_{max}	ϵ'_{max}	$kf\epsilon'd\nu$[b]
I	1	324	1620	115
II	2	313	1330	114
III	3	310	1480	100
IV	2 (*cis*)	312	2420	155
V	2 (*trans*)	308	2470	168

[a] $\epsilon' = \epsilon$(compound) $- [\epsilon(4\text{-}NO_2C_6H_4CH_3) + \epsilon(4\text{-}NH_2C_6H_4CH_3)]$. [b] Relative integrated intensity from 280 to 650 mμ.

Despite the great differences in the orientations of the donor and acceptor rings in these molecules, all showed the spectral characteristics of molecular complexes–increased absorption intensity over that expected and an absorption shift toward the visible.[2,3] Therefore, the geometrical orientation of the donor and acceptor in a molecular complex is not critical in determining whether charge-transfer interaction will occur.[4]

If the maximum in the anomalous absorption corresponds to the charge-transfer band, then the prediction of Orgel and Mulliken[1] regarding the intensities (variable) and the wave lengths (relatively invariant) of this band for complexes with different orientations of donor and acceptor is firmly supported by this work.

(2) L. J. Andrews, *Chem. Revs.*, **54**, 713 (1954).

(3) Studies at different concentrations gave the same results indicating the anomalies must be attributed to intramolecular rather than intermolecular interactions.

(4) Direct charge-transfer interaction of nitro and amino groups, sometimes suggested for nitroaromatic-amine complexes, is very improbable here. The rings must interact.

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RECEIVED APRIL 6, 1959

MECHANISM OF FORMATION OF ISOPENTENYL PYROPHOSPHATE

Sir:

During the enzymatic synthesis of squalene the carboxyl groups of six mevalonic acid (MVA) molecules are eliminated.[1] When this reaction is allowed to take place in D_2O, approximately 4 atoms of D, or less than 1 atom per molecule of MVA, are incorporated into the hydrocarbon.[2] We have interpreted this result as showing that decarboxylation occurs without protonation of the carbon chain, that it is concerted with the elimination of OH (or OR) from C-3 of MVA and that the reaction product is a derivative of Δ^3-isopentenol, (3-methyl-3-butenol-1).[2] With the identification of isopentenylpyrophosphate[3,4] as the condensing unit in squalene synthesis, these conclusions have been greatly strengthened. We now wish to present more direct evidence for the concerted nature of the decarboxylation process. A yeast enzyme, approximately 100-fold purified,[5] catalyzes the irreversible transformation: MVA-5-pyrophosphate[3] + ATP → isopentenylpyrophosphate + CO_2 + ADP + P_i. All four products are formed in stoichiometric amounts (Table I). Furthermore, kinetic experiments show that CO_2 evolution and ADP formation occur at identical rates and without a lag period. When T_2O is present during this reaction the isopentenyl moiety of the isolated isopentenylpyrophosphate is essentially free of T (T:C^{14} ratios in two experiments 0.010 and 0.040) demonstrating that the above reaction occurs without protonation of the carbon chain.[6] It is established by this finding and by the synchronous appearance of the products that the reaction of the substrate with ATP, the removal of the OH function and the decarboxylation of the MVA ester cannot be separate, consecutive events. Since ADP and P_i are formed from ATP in stoichiometric amounts and since the elements of ATP are not found in the reaction product,[5] the substrate must be phosphorylated and the same phosphate residue again eliminated in the course of the reaction.

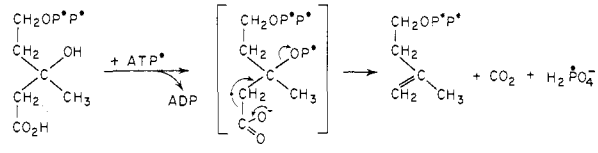
TABLE I

STOICHIOMETRY OF PRODUCTS FORMED IN THE ENZYMATIC DECARBOXYLATION OF MVA-PYROPHOSPHATE TO ISOPENTENYLPYROPHOSPHATE

The enzyme was obtained from yeast autolysate[3] by steps including precipitation with $(NH_4)_2SO_4$ (45–68% saturation), and with ethanol (13–35%), and chromatography on diethylaminoethyl cellulose column.[5] The incubation system contained $MnSO_4$ 0.004 *M*, phosphate buffer, *p*H 7, 0.04 *M*, ATP^{32} 0.0002 *M* and 1.5 mg. of enzyme in a total volume of 2.5 ml. The reaction products were separated by chromatography on Dowex-1 formate.

	μ moles
C^{14}-MVA-pyrophosphate added	0.41
Isopentenylpyrophosphate formed	.40
$C^{14}O_2$[a]	.39
ADP formed	.44
Inorganic P	.44

[a] The substrate in this flask was 1-C^{14} MVA pyrophosphate incubated under the same conditions as above in stoppered Warburg flasks. $C^{14}O_2$ was absorbed in KOH and precipitated as $BaCO_3$.



(1) P. A. Tavormina and M. H. Gibbs, THIS JOURNAL, **78**, 6210 (1956).

(2) H. Rilling, T. T. Tchen and K. Bloch, *Proc. Nat. Acad. Sci. (U. S.)*, **44**, 167 (1958).

(3) S. Chaykin, J. Law, A. H. Phillips, T. T. Tchen and K. Bloch, *ibid.*, **44**, 998 (1958).

(4) F. Lynen, H. Eggerer, U. Henning and I. Kessler, *Angew. Chem.*, **70**, 738 (1958).

(5) K. Bloch, S. Chaykin, A. H. Phillips and A. de Waard, manuscript in preparation.

(6) These values are sufficiently small to exclude any possible masking of protonation by an isotope effect.