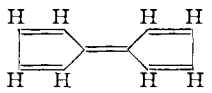

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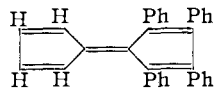
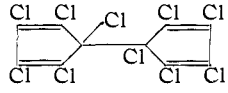
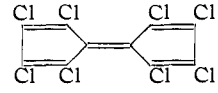


II

wherein the fulvalene nucleus was not an integral part of a fused aromatic system, as, for example, is the case in bis-(biphenylene)-ethylene.[4] However, no compound has been described wherein the fulvalene system represents the only unsaturation present. It should be noted that extensive halogen substitution generally decreases the reactivity of the diene system; for example, hexachlorobutadiene behaves much like a saturated compound.[5] We have synthesized bis-(pentachlorocyclopentadienyl), or perchloro-9,10-dihydrofulvalene, m.p. 121° (III) (λ_{max} 330 mμ, ϵ 2950), by cuprous chloride coupling of hexachlorocyclopentadiene.[6] Compound III was reduced over platinum to give bicyclopentyl, b.p. 188–190°, n^{20}D 1.4650. On being heated to 250°, III lost a mole of chlorine giving compound IV (85%), m.p. 347°. *Anal.* Calcd. for $C_{10}Cl_8$: C, 29.70; Cl, 70.30; mol. wt.,



III



IV

404. Found: C, 29.48; Cl, 70.86; mol. wt., 429 (vapor pressure depression in benzene). Ultraviolet absorption of IV (in cyclohexane) was broad and intense from 200–400 mμ, λ_{max} 267 mμ, ϵ 21,400, λ_{max} 277 mμ, ϵ 23,900, λ_{max} ~ 310 mμ (partially submerged), ϵ ~ 10,000. Hydrogenation of IV in ethanol over copper chromite gave bicyclopentyl, b.p. 60–65° (20 mm.), n^{20}D 1.4653, identical with an authentic sample prepared by sodium coupling of cyclopentyl bromide.[6] This is consistent with the behavior of III and other chlorocarbons possessing the bicyclopentyl carbon skeleton.[6] It is therefore concluded that IV is perchlorofulvalene.

We explain the absence of pronounced color in perchlorofulvalene (it is medium yellow rather than orange or red) on the grounds that steric interaction between the chlorine atoms in the 4- and 5- and the 1- and 8-positions causes rotatory distortion of the 9–10 ethylenic bond forcing the molecule into a "warped" conformation. Our calculations indicate an interference of chlorine radii of about 0.5 Å. The resultant non-planarity would disturb inter-ring resonance in much the same manner as in the *o,o,o′,o′*-substituted biphenyls[7,8] and would depress lower energy light absorption (in the visible region). Riemschneider[9] has claimed the synthesis of perchlorofulvalene by zinc-acid reductive coupling of hexachlorocyclopentadiene. In view of a recent report[10] it is now apparent that the product described by Riemschneider was actually 1,2,3,4-tetrachlorocyclopentadiene, m.p. 62°.

Because of heavy halogen substitution, IV is not a highly reactive compound. In contrast to II, IV does not take part in the Diels–Alder reaction. Under forcing conditions, both III and IV add chlorine to give a rearranged product, perchloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (V), m.p. 220–221°, λ_{max} 224 mμ, ϵ 15,800[6].

(4) P. Karrer, "Organic Chemistry," Fourth English Edition, Elsevier Publishing Co., New York, N. Y., 1950, p. 104.

(5) E. H. Huntress, "Organic Chlorine Compounds," John Wiley and Sons, New York, N. Y., 1948, p. 871.

(6) E. T. McBee, J. D. Idol, Jr., and C. W. Roberts, THIS JOURNAL, **77**, 4375 (1955).

(7) L. W. Pickett, G. F. Walter and H. France, *ibid.*, **58**, 2296 (1936).

(8) R. L. Shriner, R. Adams and C. S. Marvel in Gilman's "Organic Chemistry, An Advanced Treatise," Second Edition, John Wiley and Sons, New York, N. Y., 1943, Vol. I, p. 353 *et seq.*

(9) R. Riemschneider, *Z. Naturforschung*, **6B**, 463 (1951).

(10) E. T. McBee, R. K. Meyers and C. F. Baranauckas, THIS JOURNAL, **77**, 86 (1955).

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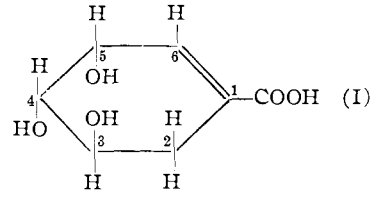
RECEIVED JULY 13, 1955

THE ENZYMATIC SYNTHESIS OF SHIKIMIC ACID FROM D-ERYTHROSE-4-PHOSPHATE AND PHOSPHOENOLPYRUVATE[1,2,3]

Sir:

Previous investigations on the biosynthesis of shikimic acid (SA) (I) from labeled glucose by



(I)

intact *Escherichia coli* have indicated that the carboxyl and carbon atoms 1 and 2 of SA are derived from a 3-carbon intermediate of glycolysis, and carbons 3, 4, 5 and 6 from the pentose-sedoheptulose pathway.[4] Furthermore, in cell-free extracts SA or its precursor, DHS, was formed from fructose-6-phosphate or FDP in a 5% yield[5,6] but from SDP almost quantitatively.[7,6] We have now found that E-4-P[8] plus PEP are rapidly and quantitatively converted to DHS (Table I). The present results also indicate that the utilization of SDP is due to its prior conversion to E-4-P and PEP (Table I).

(1) This work was supported by grants from the National Institutes of Health, U. S. Public Health Service, and the Williams–Waterman Fund.

(2) Abbreviations: SA, shikimic acid; DHS, 5-dehydroshikimic acid; E-4-P, D-erythrose-4-phosphate; PEP, phosphoenolpyruvate; SDP, sedoheptulose-1,7-diphosphate; FDP, fructose diphosphate; DHAP, dihydroxy-acetone phosphate; 3-PGA, D-3-phosphoglyceric acid; DPN, diphosphopyridine nucleotide; P_i, inorganic phosphate.

(3) For a review of the role of DHS and SA in the biosynthesis of the aromatic amino acids see B. D. Davis in "Amino Acid Metabolism" (W. D. McElroy and B. Glass, eds.), The Johns Hopkins Press, Baltimore, Md., 1955, pp. 799–811.

(4) P. R. Srinivasan, H. T. Shigeura, M. Sprecher, D. B. Sprinson, and B. D. Davis, manuscript in preparation; D. B. Sprinson, in "Amino Acid Metabolism", (W. D. McElroy and B. Glass, eds.), The Johns Hopkins Press, Baltimore, Md., 1955, pp. 817–825.

(5) E. B. Kalan, B. D. Davis, P. R. Srinivasan and D. B. Sprinson, manuscript in preparation.

(6) Certain of these results appeared in preliminary form (E. B. Kalan and P. R. Srinivasan, in "Amino Acid Metabolism" (W. D. McElroy and B. Glass, eds.), The Johns Hopkins Press, Baltimore, Md., 1955, pp. 826–830).

(7) P. R. Srinivasan, D. B. Sprinson, E. B. Kalan and B. D. Davis, manuscript in preparation.

(8) C. E. Ballou, H. O. L. Fischer, and D. L. MacDonald, THIS JOURNAL, **77**, 2658 (1955).

TABLE I

SYNTHESIS OF DHS FROM E-4-P + PEP AND FROM SDP

Cell-free extracts were prepared by subjecting cells of freshly harvested *E. Coli* mutant 83-24 (B. D. Davis, *J. Biol. Chem.*, **191**, 315 (1951)) to ultrasonic vibration. The incubation mixtures contained 0.1 ml. of extract (2 mg. of protein), 5 μM of $MgCl_2$, 50 μM of $PO_4^{=}$ buffer *p*H 7.4, 0.25 μM of E-4-P + 0.3 μM of PEP, *or* 0.25 μM of SDP, + additions (10 μM of KF, or 0.5 μM of iodoacetate) in a final volume of 1 ml. (When iodoacetate was added, the solution, 0.95 ml., was preincubated at 37° for 15 minutes prior to the addition of substrate.) Following incubation at 37° for the indicated length of time aliquots were removed for the bioassay of DHS with *Aerobacter aerogenes* mutant A170-143S1 (B. D. Davis and U. Weiss, *Arch. Exp. Path. and Pharm.*, **220**, 1 (1953)).

Substrates and additions	Per cent. conversion 1 hour	2 hours
E-4-P + 0.3 μM PEP	88	86
+ fluoride + 0.3 μM PEP	88	88
+ fluoride + 0.5 μM 3-PGA	0	0
+ iodoacetate + 0.3 μM PEP	90	90
+ iodoacetate + 0.5 μM 3-PGA	90	90
SDP	39	83
+ fluoride	0	0
+ fluoride + 0.5 μM FDP	0	0
+ fluoride + 0.5 μM 3-PGA	0	0
+ fluoride + 0.5 μM pyruvate	0	0
+ fluoride + 0.3 μM PEP	37	80
+ iodoacetate	0	0
+ iodoacetate + 0.5 μM FDP	0	0
+ iodoacetate + 0.5 μM 3-PGA	46	83
+ iodoacetate + 0.5 μM pyruvate	0	0
+ iodoacetate + 0.3 μM PEP	46	83

It may be seen from Table I that the synthesis of DHS from E-4-P and PEP was not inhibited by fluoride or iodoacetate, while that from SDP was completely inhibited. The reversal of the fluoride inhibition by PEP, and of the iodoacetate inhibition by either 3-PGA or PEP, suggests that the glycolysis reactions from triose phosphate to PEP are involved in the conversion of SDP to DHS. The most reasonable explanation of these results is based on the series of reactions

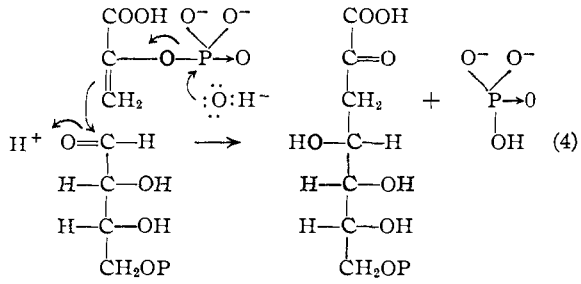
$$\text{SDP} \rightleftharpoons \text{E-4-P} + \text{DHAP} \qquad (1)^9$$

$$\text{DHAP} \rightleftharpoons \text{3-PGA} \rightleftharpoons \text{PEP} \qquad (2)$$

$$\text{E-4-P} + \text{PEP} \longrightarrow \longrightarrow \text{DHS} \qquad (3)$$

The expected requirement for DPN in (2) is shown by the observation (not reported in the table) that charcoal treated extracts do not convert SDP to DHS unless DPN is added. On the other hand, these extracts can carry out the synthesis of DHS from E-4-P and PEP without added DPN, as would be expected from the fact that the reactants and products of (3) are on the same level of oxidation.[10]

The first reaction in (3) may be postulated as the condensation of E-4-P and PEP (Reaction 4). This condensation resembles an aldolase type of



(4)

reaction, but is more closely analogous to the CO_2 fixation reaction, PEP + CO_2 + OH^- → oxaloacetate + P_i.[11] The product is assumed to be 2-keto-3-deoxy-7-phospho-D-*gluco*heptonic acid, in which carbons 4 and 5 have the same configuration as carbons 3 and 4 of DHS. The inversion of the configuration of carbon 4 of SDP during its conversion to DHS would then be a result of the cleavage and recondensation postulated in Reactions 1-3. Further studies of the reactions and intermediates involved in the synthesis of DHS from E-4-P + PEP are in progress.

It is a pleasure to acknowledge the hospitality and generosity accorded to us by Dr. B. L. Horecker for the enzymatic preparation of SDP.[9] We are indebted to Dr. B. D. Davis for the mutant strains used in this work, to Mr. W. E. Pricer, Jr., for PEP, and to Dr. C. E. Ballou for E-4-P.

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RECEIVED JULY 18, 1955

(9) B. L. Horecker, P. Z. Smyrniotis, H. H. Hiatt, and P. A. Marks, *J. Biol. Chem.*, **212**, 827 (1955).

(10) Charcoal treated extracts were unable, however, to synthesize DHS from SDP and PEP, with or without added fluoride. Curiously, the activity was completely restored by DPN, even after preincubation with iodoacetate. It would appear that, under these experimental conditions, the ability of SDP to serve as a source of E-4-P for condensation with added PEP is dependent on still unknown factors. This observation is being further investigated.

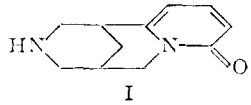
(11) R. S. Bandurski and C. M. Greiner, *J. Biol. Chem.*, **204**, 781 (1953); T. T. Chen and B. Vennesland, *ibid.*, **213**, 533 (1955).

(12) Established Investigator, American Heart Association.

THE SYNTHESIS OF *dl*-CYTISINE

Sir:

As a consequence of the admirable investigations of Partheil,[1] Freund,[2] Ing,[3] Späth[4] and others, the structure I has been established for cytisine, the



I

lupin alkaloid present in the very poisonous laburnum as well as in many other genera of Leguminosae, *e.g.*, *Cytisus*. Although sparteine, the well-known tetracyclic lupinane, has yielded to synthesis,[5] the cytisine system—unsymmetrical and partially aromatic as well as bridged—has up until now withstood attempts at construction in the laboratory. The operations outlined below constitute the total synthesis of the racemic form of this unusual base.

2(α-Pyridyl)-allylmalonic acid (II), m.p. 115°

(1) A Partheil, *Arch. Pharm.*, **232**, 161 (1894).

(2) M. Freund, *Ber.*, **37**, 22 (1904).

(3) H. R. Ing, *J. Chem. Soc.*, 2778 (1932).

(4) E. Späth and F. Galinovsky, *Ber.*, **65**, 1526 (1932).

(5) (a) N. J. Leonard and R. E. Beyler, THIS JOURNAL, **70**, 2298 (1948); (b) G. R. Clemo, R. Raper and W. S. Short, *Nature*, **162**, 296 (1948); (c) F. Šorm and B. Keil, *Collection Czechoslov. Chem. Commun.*, **13**, 544 (1948); (d) F. Galinovsky and G. Kainz, *Monatsh.*, **80**, 112 (1949).