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 $PO_4^{\equiv}$ 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

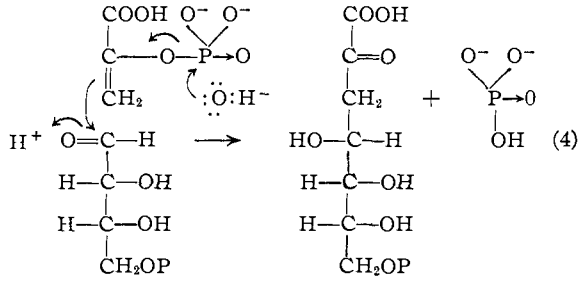
$$\text{SDP} \rightleftharpoons \text{E-4-P} + \text{DHAP} \qquad (1)^9$$

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

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

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.

DEPARTMENT OF BIOCHEMISTRY P. R. SRINIVASAN
COLLEGE OF PHYSICIANS AND SURGEONS M. KATAGIRI
COLUMBIA UNIVERSITY DAVID B. SPRINSON[12]
NEW YORK 32, NEW YORK

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.

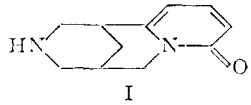
(11) R. S. Bandurski and C. M. Greiner, *J. Biol. Chem.*, **204**, 781 (1953); T. T. Chen and B. Vennesland, *ibid.*, **213**, 533 (1955).

(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).

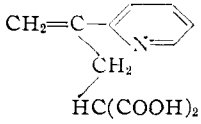
(2) M. Freund, *Ber.*, **37**, 22 (1904).

(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).

(dec.) (Found: C, 59.66; H, 5.13), secured by saponification of the diethyl ester which results from interaction of sodio malonic ester with 2-(α-pyridyl)-allyl acetate,[6] was condensed with benzylamine and formaldehyde; the Mannich reaction, accompanied by decarboxylation and cyclization, yielded N-benzyl-3-(α-pyridyl)-piperidine-5-carboxylic acid, which was converted without purification to the ethyl ester,[7] b.p. 183–184° (0.1 mm.) (Found: 3, 74.45; H, 7.84). Lithium aluminum hydride reduction afforded N-benzyl-3-

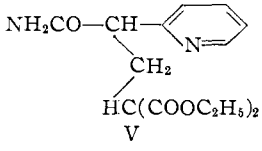


II

IV

(α-pyridyl)-5-methylolpiperidine (III),[7] b.p. 193–194° (0.1 mm.) (Found: C, 76.78; H, 8.24; N, 9.84). Refluxing III with hydrobromic acid led to the 5-bromomethylpiperidine, which, as the free base, was quaternized without isolation to the tricyclic pyridinium bromide IV, m.p. 170.5–171.5° (Found: C, 62.80; H, 6.29; N, 8.23). The salt, on oxidation with alkaline ferricyanide, gave rise to *dl*-N-benzylcytisine, m.p. 137.5–139° (Found: C, 77.16; H, 7.22), the infrared spectra (obtained in chloroform and carbon disulfide) of which were identical in every detail with the corresponding spectra of N-benzylcytisine, m.p. 140–142° (Found: C, 77.23; H, 7.39), obtained by monoalkylation of the alkaloid. Hydriodic acid cleavage[8] of *dl*-N-benzylcytisine afforded *dl*-cytisine, m.p. 145–146° (Found: C, 69.53; H, 7.46); the infrared spectra of the synthetic base and natural alkaloid were indistinguishable.

The key intermediate III was also attained by an alternate series of steps. The addition of α-pyridylacetamide, m.p. 119–120° (Found: C, 62.06; H, 6.19), to methylenemalonic ester yielded the Michael product (V), m.p. 106° (Found: C, 58.15; H, 6.57), which was cyclized by means of sodium methoxide to 3-(α-pyridyl)-5-carboethoxy-



V

glutarimide, m.p. 136–138° (Found: C, 59.65; H, 5.81). Alkylation of the glutarimide, accomplished by heating the sodium salt with benzyl chloride, followed by lithium aluminum hydride reduction, gave rise to: (1) N,5-dibenzyl-3-(α-pyridyl)-5-methylolpiperidine, m.p. 146–147° (Found: C, 80.52; H, 7.51; N, 7.69), resulting from dialkylation, and (2) the desired alcohol III, identified by boiling point, infrared spectral comparison, and conversion to IV.

Since the alkaloids caulophylline (N-methylcytisine)[9] and rhombifoline (N-but-3-enylcytisine)[8] have been previously obtained by alkylation of cytisine,[8,10] the synthetic route described above thereby embraces these natural products as well.

Acknowledgment.—This work was supported by grants from the Research Committee of the Wisconsin Alumni Research Foundation and from the National Science Foundation. The authors wish to express their gratitude to Professors Marion and Galinovsky for gifts of cytisine.

(6) F. Bohlmann, N. Ottawa and R. Keller, *Ann.*, **587**, 162 (1954).

(7) The ratio of *cis* and *trans* isomers was not determined.

(8) W. F. Cockburn and L. Marion, *Can. J. Chem.*, **30**, 92 (1952).

(9) J. U. Lloyd, *Proc. Am. Pharm. Assoc.*, **41**, 115 (1893); F. B. Power and A. H. Salway, *J. Chem. Soc.*, **103**, 191 (1913).

(10) A. Partheil, *Ber.*, **24**, 635 (1891).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN EUGENE E. VAN TAMELEN
MADISON, WISCONSIN JOHN S. BARAN

RECEIVED JUNE 17, 1955

NUCLEAR MAGNETIC RESONANCE SPECTRA OF THE CONDENSED PHOSPHATES

Sir:

As part of a general study of the chemical shifts in the nuclear magnetic resonance spectra[1] of phosphorus compounds, some observations were made which give fundamental support to the idea previously developed,[2,3] that in the phosphates there is a difference between isolated, end, middle, and branching-point PO_4 groups. These four kinds of PO_4 groups each give a separate resonance peak in the nuclear magnetic resonance spectrum. When the chemical shift is measured in parts per million of the applied magnetic field (7140 gauss) and phosphorus tribromide is used as the reference standard, the positions given in Table I are found for the resonance peaks of various types of PO_4 groups.

TABLE I

Type of PO_4 group	Chemical shift[a] p.p.m.
Orthophosphate ions (isolated groups)	
trisubstituted (normal) salts	233
1 to 3 hydrogens/P atom	238
End groups	
doubly substituted (no H^+ ions)	244
1 to 2 hydrogens/P atom	247
Middle groups	
short chains	256
long chains	259
Branching points	
alkali metal ultraphosphates	268
azeotropic phosphoric acid[6]	272

[a] The chemical shifts of the various phosphates are measured to *ca.* ± 2 p.p.m. with respect to each other. The actual measurements were made relative to 85% orthophosphoric acid; however, the values reported are referred to phosphorus tribromide as the zero reference and were obtained by adding the value of the phosphorus tribromide shift, relative to orthophosphoric acid, to each result.

Examination of the spectrum of sodium tripolyphosphate solutions shows two peaks, one for the end groups and one for the middle groups.

(1) E. M. Purcell, *Science*, **118**, 431–436 (1953); F. Bloch, *Science*, **118**, 425–430 (1953); Varian Associates, Palto Alto, Calif., "*Radio-frequency Spectroscopy*," **1**, No. 1 (1953), No. 2 (1954) and No. 3 (1955).

(2) J. R. Van Wazer and K. A. Holst, THIS JOURNAL, **72**, 639 (1950); J. R. Van Wazer, *ibid.*, **72**, 647 (1950); *ibid.*, paper X in press; and "Encyclopedia of Chemical Technology" (Kirk and Othmer, editors), Interscience, New York, 1953, Vol. X, pp. 403–429, 469–472.

(3) J. R. Van Wazer, THIS JOURNAL, **72**, 644 (1950).