seemed somewhat low, based on the toxicity of β-cyanoalanine to the rat,[6] to warrant serious consideration of this amino acid as an active factor in lathyrism. We now wish to report that common vetch contains, as well, significant quantities of β-cyano-L-alanine in a bound form which can also be neurotoxic. Moreover, observations with a second species indicate that both the seed of common vetch and its active principles can be far more toxic than experience with the rat had suggested.

Preliminary analytical procedures allowed characterization of the bound β-cyanoalanine as the acidic dipeptide glutamyl-β-cyanoalanine (R_f 0.59, pyridine–water 65:35). The β-cyanoalanine peptide was then isolated from common vetch by chromatography of material in 30% ethanol seed extracts directly on Dowex 1-X4 with pyridinium acetate buffer, pH 4.1, as well as by preparative electrophoresis on blocks of Solka-floc at pH 5.7 and pH 3.5. The purified peptide was then crystallized from water–ethanol, yield 59%, m.p. 185.5–186° dec. (*Anal.* Calcd. for $C_9H_{13}N_3O_5$: C, 44.4; H, 5.39; N, 17.3. Found: C, 44.4; H, 5.52; N, 17.0). Its *dicyclohexylammonium (DCHA) salt* melts at 184–185° dec.; $[\alpha]^{25}D$ +15.1° (*c* 0.6, 2.5% $KHCO_3$). *Anal.* Calcd. for $C_{21}H_{36}N_4O_5 \cdot 0.5\ H_2O$: C, 58.2; H, 8.60; N, 12.9; H_2O, 2.08. Found: C, 58.1; H, 8.46; N, 13.1; H_2O, 2.61. Hydrolysis in 6 *N* HCl for 10 hr. at 110° followed by analysis on the Beckman automatic amino acid analyzer[8] gave a quantitative yield of aspartic acid, glutamic acid, and ammonia, all in a molar ratio to each other of 1:1. On treatment[6] of the DCHA salt with sodium in ammonia containing methanol followed by desalting, hydrolysis, and analysis, less than 1% of aspartic acid was found. Glutamic acid and 2,4-diaminobutyric acid, which were present in molar ratio of 1:1, were obtained in 89% yield.

The isolated peptide was established to be N-(γ-L-glutamyl)-β-cyano-L-alanine through synthesis of the latter, accomplished by hydrogenolysis in the presence of a palladium catalyst of the intermediate carbobenzoxy-γ-L-glutamyl-β-cyano-L-alanine α-benzyl ester which was prepared in 69% yield by coupling carbobenzoxy-L-glutamic acid α-benzyl ester and β-cyano-L-alanine by the mixed anhydride procedure with isobutyl chlorocarbonate. Synthetic γ-L-glutamyl-β-cyano-L-alanine, which possessed the expected elementary composition, agreed with the isolated peptide in melting point, and admixture of them, as well as of their DCHA salts, caused no depression in the respective melting points. Optical rotations and infrared spectra of the DCHA salts were the same for both substances. The two materials showed identical behavior on chromatography on paper in two systems as well as on Dowex 1-X4 (acetate) and Amberlite CG-120 (H^+) ion-exchange resin columns, and had the same electrophoretic mobility on paper at pH 5.7 or 8.6. Moreover, the natural and synthetic peptides were readily distinguishable chromatographically and electrophoretically from the product of hydrogenolysis of carbobenzoxy-α-L-glutamyl-β-cyano-L-alanine γ-benzyl ester prepared in a similar way from carbobenzoxy-L-glutamic acid γ-benzyl ester. The natural and synthetic γ-L-glutamyl-β-cyano-L-alanines showed similar toxicities when injected subcutaneously into young rats.

Isolated γ-L-glutamyl-β-cyano-L-alanine is similar in potency on a molar basis to β-cyano-L-alanine when administered to male weanling Sherman rats subcutaneously, or as a single dose by stomach tube. In White Leghorn chicks the dipeptide, when administered subcutaneously or *per os*, is approximately half as toxic as β-cyano-L-alanine. The concentration of γ-glutamyl-β-cyanoalanine is 0.58% in the seed of common vetch and rises to 1.67 to 2.6% (dry wt.) in the young seedling, suggesting the latter stage of development is potentially more toxic than the seed. A mixture of γ-L-glutamyl-β-cyano-L-alanine and β-cyano-L-alanine, incorporated into a basal ration at half the concentration (0.29 and 0.075%) at which these occur in the seed, has an effect in young chicks similar to a 50% common vetch seed ration, the two diets producing within 6 and 6.5 days (average), respectively, terminal convulsive states with a characteristic opisthotonus.[9]

There has been some question of the neurotoxicity of common vetch seed,[7] and in view of seeming conflicting reports in the literature[10-12] we wish to note that toxicity varies strikingly in different species. In general confirmation of recently reported studies with the chick,[13] diets incorporating 20 to 50% of common vetch seed have been found highly neurotoxic to this species, whereas, in our earlier experiments with the rat, similar concentrations of vetch had appeared nontoxic. As in other experiments,[11] even diets very high in vetch (85 and 100%), although tending to retard growth, produced no obvious neurotoxicity in the rat. Considerable species difference holds also for β-cyano-L-alanine, which, in the diets employed, has been found neurotoxic to the chick near the 0.075% level (average survival 10.5 days) in contrast to the rat which tolerates more than 9 times this concentration.[6] On the basis of the concentrations of γ-L-glutamyl-β-cyano-L-alanine and β-cyano-L-alanine in the seed of common vetch and the now established levels of toxicity of these two principals in the two species, one should indeed expect diets which contain 20 to 100% of common vetch seed to be highly neurotoxic to the chick while they could be nontoxic to the rat. The advisability of using diverse species when testing suspect foods or chemicals for neurotoxicity is evident.

These studies constitute the first report of the natural occurrence of β-cyanoalanine in peptide or bound form and also suggest that γ-glutamyl-β-cyanoalanine and β-cyanoalanine are probably the chief neurotoxic principals of common vetch seed.

(8) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(9) The basal ration was commercial Wayne High Fiber Pullet Developer obtained from Allied Mills, Inc., Chicago, Illinois. It contained minimum 14% protein, 2.5% fat, and 9% fiber. The test diets used for the vetch and the isolated principles may not be comparable nutritionally. The isolations were followed chiefly by chemical means due to the lack of a sensitive bioassay at the start of this work.

(10) H. Stott, *Indian J. Med. Res.*, **18**, 51 (1930–1931).

(11) R. McCarrison, *ibid.*, **15**, 797 (1927–1928).

(12) L. A. P. Anderson, A. Howard, and J. L. Simonsen, *ibid.*, **12**, 613 (1924–1925).

(13) J. A. Harper and G. H. Arscott, *Poultry Sci.*, **41**, 1968 (1962).

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Isolation and Reactions of a Stable Enol Phosphonium Salt

Sir:

Enol phosphonium salts I have been postulated[1-3] as intermediates in the Perkow[4] and halogen migration[1]

(1) A. J. Speziale and R. C. Freeman, *J. Am. Chem. Soc.*, **82**, 1903 (1960); A. J. Speziale and L. R. Smith, *ibid.*, **84**, 1868 (1962).

(2) M. S. Kharasch and I. S. Bengelsdorf, *J. Org. Chem.*, **20**, 1356 (1955).

(3) I. J. Borowitz and L. I. Grossman, *Tetrahedron Letters*, 471 (1962).

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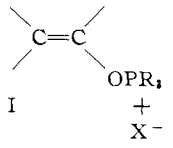
reactions of halocarbonyl compounds with tertiary phosphines and phosphites.



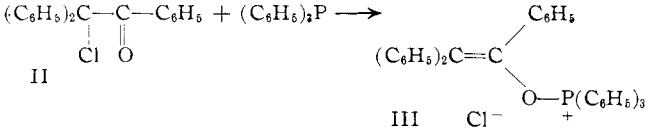
I

R = alkyl, aryl, or alkoxy

The formation of this intermediate can occur by attack of trivalent phosphorus on carbonyl carbon,[1,2] carbonyl oxygen,[1,5] or on halogen.[1,6] Although the position of attack has not yet been unequivocally determined, halogen attack seems the most likely at this time.[1]

In two instances[5,6] the isolation of an enol phosphonium salt was reported, but essentially no physical data were given in support.

We now wish to report the isolation and characterization of an enol phosphonium salt; that derived by the action of triphenylphosphine on the haloketone II. The salt III was obtained in good yield (68%). It is an

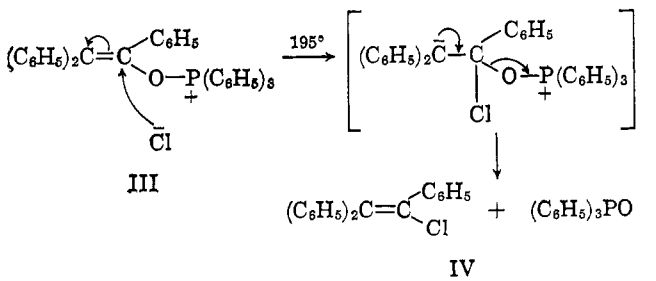


II III

extremely hygroscopic white solid, insoluble in refluxing xylene and having the correct elemental analysis for III. *Anal.* Calcd.: C, 80.28; H, 5.28; P, 5.46; Cl, 6.16. Found: C, 80.85; H, 5.49; P, 5.48; Cl, 6.18; ionic Cl, 6.16. The infrared spectrum [($CHCl_3$): 3.01 μ (w), 3.40 (m), 4.16 (w), 6.30 (m), 6.74 (w), 9.96 (m), 8.58 (m), 8.94 (s), 9.63 (m), 9.86 (s), 10.05 (s), 10.34 (s)] showed no carbonyl absorption. Proton magnetic resonance analysis showed a multiplet centered at 2.6 τ. The P^{31} n.m.r. spectrum exhibited a chemical shift of -63 p.p.m. relative to 85% phosphoric acid.

Recrystallization of III from ethylene dichloride afforded white needles, which analyzed for a 1:1 adduct of III and ethylene dichloride. *Anal.* Calcd.: C, 71.86; H, 5.09; P, 4.64; Cl, 15.94; ionic Cl, 5.31. Found: C, 71.92; H, 5.20; P, 4.76; Cl, 15.90; ionic Cl, 5.68. The n.m.r. spectrum exhibited a multiplet at 2.6 τ and a singlet at 6.24 τ. The ratio of the peak areas was 8.2:1. Ethylene dichloride alone exhibits a singlet at 6.26 τ.

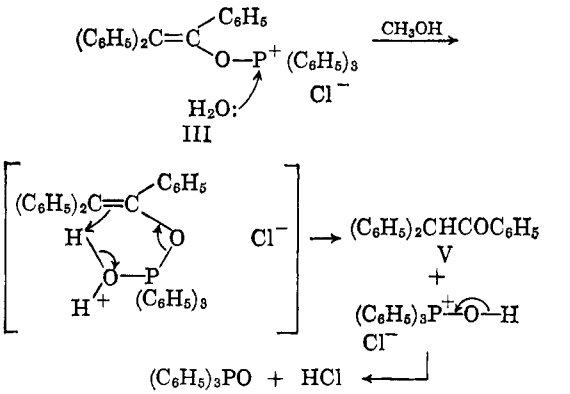
Pyrolysis of III was carried out at 195° (0.2 mm.) in a sublimation apparatus. The products of the pyrolysis were triphenylphosphine oxide and the expected product of the halogen migration reaction, triphenylchloroethylene (IV). The melting point of IV was not depressed



III IV

upon admixture of authentic material prepared by the route of van de Kamp.[7] The infrared spectra were superimposable.

Hydrolysis of III led to triphenylphosphine oxide and the dehalogenated ketone V. Again the melting point was not depressed upon admixture of authentic material, and the infrared spectra were superimposable.



III V

The elucidation of the mechanism of the decomposition of these salts *via* the Perkow and halogen migration reactions is now continuing.

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(5) S. Trippett, *J. Chem. Soc.*, 2337 (1962).
(6) H. Hoffmann and H. J. Diehr, *Tetrahedron Letters*, 583 (1962).
(7) J. van de Kamp and M. Sletzinger, *J. Am. Chem. Soc.*, **63**, 1879 (1941).

Steroids. CCXLIII.[1] Steroids of Unnatural Configuration. The Synthesis of 9β,10α-19-Nor Steroids

Sir:

Retro (9β,10α) steroids have so far been obtained only by photochemical reactions.[2] We wish to report a chemical synthesis of two 19-nor steroids with the *anti-cis-anti-trans* stereochemical arrangement depicted in IVa,b. It is well known[3] that Birch reduction of estrone methyl ether (9α-H) and subsequent acid treatment leads to 19-nortestosterone (9α,10β stereochemistry). However, conformational considerations indicated that an analogous reduction of a ring A aromatic (9β-H) steroid should lead to a 19-nor-Δ^4-3-ketone with 9β,10α-stereochemistry.

An approach to 9β-ring A aromatic steroids arose from studies with 11-keto ring A aromatic pregnanes.[4] Alkaline hydrolysis (1% sodium hydroxide in methanol) of 3-hydroxy-19-norpregna-1,3,5(10)-triene-11,20-dione benzoate (Ib)[4] afforded a phenol Id [m.p. 202–204°; [α]D +248° (dioxan); λ_{max}^{EtOH} 282 mμ (log ϵ 3.31). *Anal.* Found: C, 76.65; H, 7.45; O, 15.47] which was isomeric with but different from the expected product Ia.[4] The phenol Id was characterized as its benzoate Ie [m.p. 179–181°; [α]D +204° ($CHCl_3$); λ_{max}^{EtOH} 231 mμ (log ϵ 4.34). *Anal.* Found: C, 78.19; H, 6.84] and methyl ether If [m.p. 157–159°; [α]D +274° ($CHCl_3$); λ_{max}^{EtOH} 280 mμ (log ϵ 3.31). *Anal.* Found: C, 77.74; H, 8.34; O, 14.29]. The optical rotatory dispersion curves of the six compounds Ia–If clearly demonstrated that the pregnane side chain was β-

(1) Part CCXLII: A. D. Cross and P. Crabbé, *Tetrahedron* (submitted for publication).
(2) (a) The classical work of Windaus, Heilbron, and their schools on the irradiation of ergosterol is summarized by L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 136; (b) J. Castells, E. R. H. Jones, G. D. Meakins, and R. W. J. Williams, *J. Chem. Soc.*, 1159 (1959), and related papers; (c) W. G. Dauben and G. J. Fonken, *J. Am. Chem. Soc.*, **81**, 4060 (1959); (d) E. H. Reerink, H. F. L. Schöler, P. Westerhof, A. Querido, A. A. H. Kassenaar, E. Diczfalusy, and K. C. Tillinger, *Nature*, **186**, 168 (1960); P. Westerhof and E. H. Reerink, *Rec. trav. chim.*, **79**, 771, 795, 1118 (1960); (e) R. Van Moorselaar, Ph.D. Thesis, University of Leiden (Holland), 1962.
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(4) A. Bowers, J. S. Mills, C. Casas-Campillo, and C. Djerassi, *J. Org. Chem.*, **27**, 361 (1962).