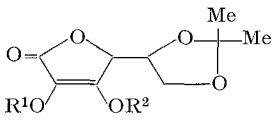
SPECIALIA

The Oxidative Dephosphorylation of Phosphoryl Esters Derived from L-Ascorbic Acid

We wish to report the synthesis and oxidative dephos phorylation of the 2- and 3-phenylphosphoryl esters [I: R^1=P(O)(OH)(OPh), R^2=H] and [I: R^1=H, R^2=P(O)(OH)(OPh)] of 5,6-isopropylidene L-ascorbic acid.



(I)

Previous work[1] on the phosphorylation of isopropylidene L-ascorbic acid [I: R^1=R^2=H][2] using phosphorus oxychloride gave, in low yield, a phosphate ester of unknown structure. In our hands, treatment of [I: R^1 = R^2 = H] with dicyclohexyl carbodiimide and monophenyl phosphoric acid in anhydrous pyridine gave a mixture of products, electrophoresis of which, at pH 5.5, indicated the presence of two phosphorus-containing compounds: each was enolic (ferric chloride/ferricyanide spray)[3].

Chromatography on DEAE cellulose, using gradient elution with triethylammonium acetate at pH 5.5, gave two compounds, A and B, the former being isolated as its barium salt (in 50% yield), and the latter as its cyclohexylammonium salt (in 25% yield). Treatment of each with diazomethane, followed by comparison of (a) proton NMR spectra and (b) the products of ozonolysis with those from [I: R^1 = R^2 = Me] and [I: R^1 = H, R^2 = Me][4], indicated that A was the 2-isomer [I: R^1 = P(O)(OPh)(OH), R^2 = H] and B was the corresponding 3-isomer of 5,6-isopropylidene L-ascorbic acid.

Since ene-diols bear a vinylogous relationship to catechols and hydroquinones, comparison with the behaviour of *ortho*- and *para*-hydroxyphenyl phosphate esters[5,6] leads one to expect that phosphate esters of ene-diols should undergo oxidative dephosphorylation. Treatment of both A and B with aqueous iodine or bromine led to a rapid liberation of monophenyl phosphoric acid. With a tenfold excess of bromine in ethanol, both A and B acted as sources for phosphoryl transfer, phenyl ethyl phosphate being produced.

The recent report[7] of analogous behaviour using the corresponding sulphate ester [I: R^1 = H, R^2 = SO_3H] is entirely in accord with our observations[8].

Zusammenfassung. Die 2- und 3-Phenylphosphorylester von 5,6-Isopropyliden L-ascorbinsäure wurden synthetisiert. Nach Oxydierung in Wasser oder Äthanol übertragen diese Ester ihre Phosphorylgruppe auf das Lösungsmittel.

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University Chemical Laboratory, Cambridge (England), April 13, 1966.

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[8] We wish to thank the Jane Coffin Childs Memorial Fund for a Fellowship (to J.W.B.H.), the University of Cambridge for the award of an I.C.I. Fellowship (to D.W.H.) and Roche Products Limited (Welwyn) for the gift of the L-ascorbic acid.
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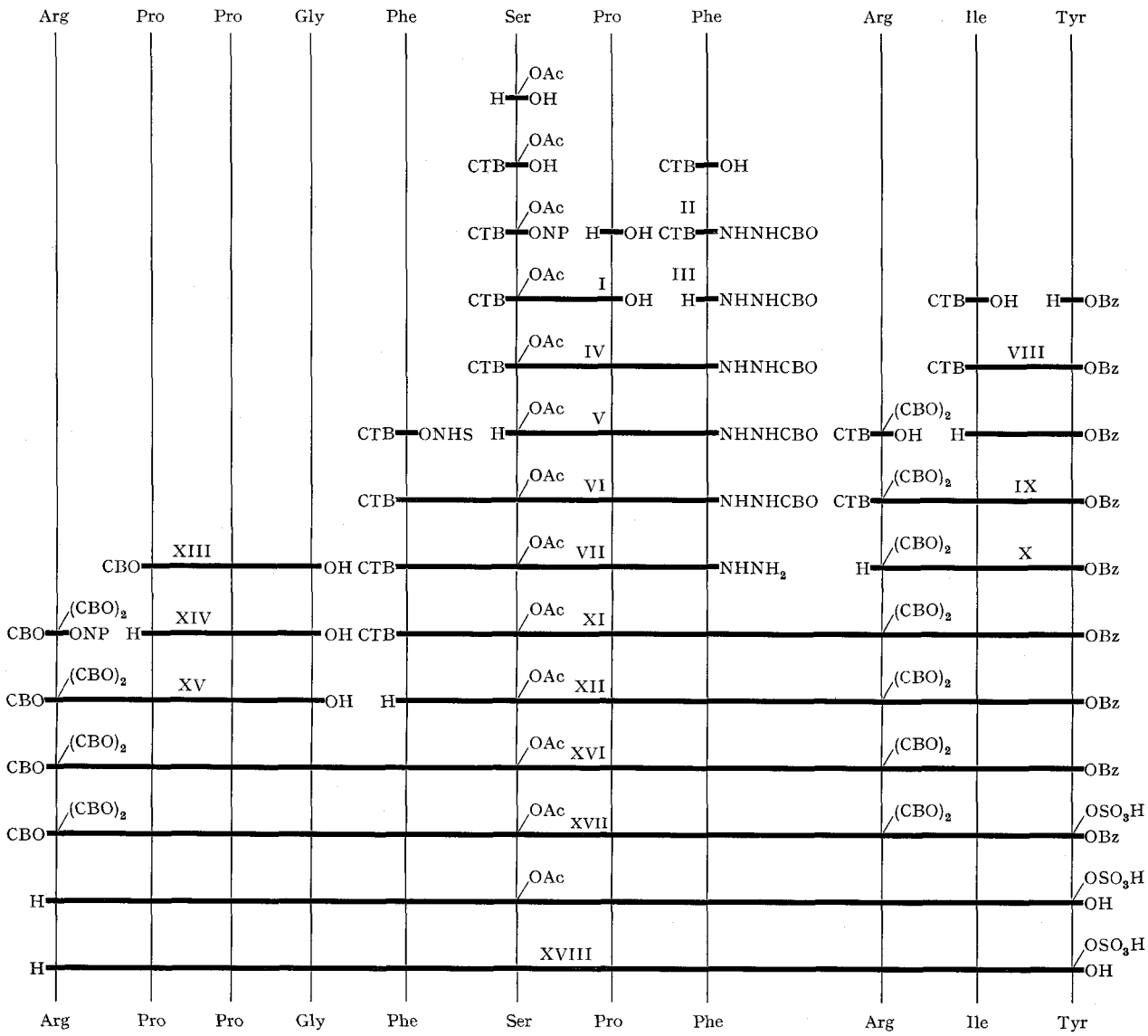
Synthesis of Phyllokinin, a Natural Bradykinin Analogue

We report the synthesis of a peptide of the formula H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Ile-Tyr(OSO_3H)-OH according to the Scheme. The product was found to be identical with phyllokinin[1,2]. Condensation of O-acetyl-serine with *p*-nitrophenyl-*ter*-butylcarbonate in DMF with one equivalent of TEA afforded N-CTB-O-acetyl-serine (50% yield; DCEA salt: m.p. 154–155°; $[\alpha]_D^{20}$ + 13°, c 1, DMF. *Anal.* Calcd. for $C_{22}H_{40}N_2O_6$: C 61.6; H 9.4; N 6.5; Found C 61.6; H 9.4; N 6.0) that, by treatment with *p*-nitrophenol and DCCI in AcOEt, afforded *p*-nitrophenyl N-CTB-O-acetyl-serinate (75% yield; m.p. 90°; $[\alpha]_D^{20}$ – 51°, c 1, DMF. *Anal.* Calcd. for $C_{16}H_{20}N_2O_8$: C 52.2; H 5.5; N 7.6; Found C 52.3; H 5.6;

[1] A. Anastasi, V. Erspamer, and J. M. Cei, Symposium on Hypotensive Peptides, Florence, Italy, October 1965.
[2] All the amino acids have the L-configuration. The following abbreviations are used throughout this paper: CBO = carbobenzyloxy; CTB = carbo-*ter*-butyloxy; TEA = triethylamine; E^a = electrophoretic mobility of a sample pre-treated with HCl/AcOH; DMF = dimethylformamide; THF = tetrahydrofurane; DCEA = dicyclohexylamine; DCCI = dicyclohexylcarbodiimide; NHS = N-hydroxy-succinimide.

N 7.6) which was condensed with proline in DMF with one equivalent of TEA to give N-CTB-O-acetyl-seryl-proline (I) (DCEA salt, 75% yield; m.p. 172–173°; $[\alpha]_D^{20}$ — 35°, c 2, DMF. *Anal.* Calcd. for $C_{27}H_{47}N_3O_7$: C 61.7; H 9.0; N 8.0. Found C 61.6; H 9.1; N 8.0). The protected hydrazide II (m.p. 114–115°; $[\alpha]_D^{20}$ — 5°, c 2, DMF. *Anal.* Calcd. for $C_{22}H_{27}N_3O_5$: C 63.9; H 6.6; N 10.2. Found C 64.0; H 6.7; N 10.2), obtained (67% yield) by condensation of N-CTB-phenylalanine and N-CBO-hydrazine in THF and in presence of DCCI, was treated with HCl/AcOH 1.3*N* to give phenylalanyl-CBO-hydrazide hydrochloride (III) (90% yield; m.p. 140°; $[\alpha]_D^{20}$ + 40°, c 2, AcOH 95%; $E_{1.9}$ = 0.76 Leu. *Anal.* Calcd. for $C_{17}H_{20}N_3O_3Cl$: C 58.4; H 5.8; N 12.0. Found C 58.2; H 5.8; N 12.0) which was treated in THF with the DCEA salt of N-CTB-O-acetyl-seryl-proline (I). After filtration of DCEA · HCl, DCCI was added and N-CTB-O-acetyl-seryl-prolyl-phenylalanyl-CBO-hydrazide (IV) was later isolated (66% yield; m.p. 175°; $[\alpha]_D^{20}$ — 52°, c 2, DMF. *Anal.* Calcd. for $C_{32}H_{41}N_5O_9$: C 60.1; H 6.5; N 11.0. Found C 60.0; H 6.5; N 11.0) and next treated with HCl/AcOH 1.3*N* to give O-acetyl-seryl-prolyl-phenylalanyl-CBO-hydrazide hydrochloride (V) (97% yield; m.p. 145°; $[\alpha_D^{20}]$ — 42°, c 1, AcOH 95%; $E_{1.9}$ = 0.57 Leu. *Anal.* Calcd. for $C_{27}H_{34}N_5O_7Cl$: C 56.3; H 6.0; N 12.2. Found C 56.6; H 6.1; N 12.0) that was treated in DMF with phenylalanine N-hydroxysuccinimidyl ester and one equivalent of TEA to give N-CTB-phenylalanyl-O-acetyl-seryl-propyl-phenylalanyl-CBO-hydrazide (VI) (83% yield; m.p. 112–115°; $[\alpha]_D^{20}$ — 52°; c 1, DMF; $E_{1.9}^a$ = 0.44 Leu. *Anal.* Calcd. for $C_{41}H_{50}N_6O_{10} \cdot {}^1/_2H_2O$: C 61.9; H 6.5; O 21.1. Found C 62.2; H 6.5; O 21.2) that, by hydrogenation (Pd/C), afforded N-CTB-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-hydrazide (VII) (72% yield; m.p. 138–141°; $[\alpha]_D^{20}$ — 44°, c 1, DMF; $E_{1.9}$ = 0.41 Leu; $E_{1.9}^a$ = 0.92 Leu. *Anal.* Calcd. for $C_{33}H_{44}N_6O_8$: C 60.7; H 6.8; O 19.6. Found C 60.5; H 7.0; O 19.7).

N-CTB-isoleucine was condensed, via the mixed anhydride (ethyl chloroformate), with tyrosine benzylester to give N-CTB-isoleucyl-tyrosine benzylester (VIII) (74% yield; m.p. 139–141°; $[\alpha]_D^{20}$ — 14°, c 0.5, DMF. *Anal.* Calcd. for $C_{27}H_{36}N_2O_6$: C 66.9; H 7.5; N 5.8. Found C 67.0; H 7.4; N 5.6) that was treated with HCl/AcOH 1.3*N* to give isoleucyl-tyrosine benzylester hydrochloride ($E_{1.9}$ = 0.66 Leu), which, without purification, was condensed in



THF with N^{α}-CTB-$N^{\omega,\omega}$-*bis*-CBO-arginine[3], via the mixed anhydride with trimethylacetic acid[3]. The resulting N^{α}-CTB-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester (IX) (85% yield; m.p. 164–166°; $[\alpha]_D^{20}$ −7°, c 0.6, DMF. *Anal.* Calcd. for $C_{49}H_{60}N_6O_{11} \cdot {}^1/_2H_2O$: C 64.1; H 6.7; N 9.2. Found C 64.3; H 6.8; N 8.8) was treated with HCl/AcOH 1.3*N* to give $N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester hydrochloride (X)[4] (yield 95%; $E_{1.9}$ = 0.66 Leu) which was condensed in DMF with CTB-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanylazide, obtained by treatment at low temperature of the hydrazide VII with HCl/isoamyl nitrite[5]. The resulting N-CTB-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester (XI)[6] (40% yield; m.p. 160–163°; $[\alpha]_D^{20}$ −25°, c 1, DMF. *Anal.* Calcd. for $C_{77}H_{92}N_{10}O_{17}$: C 64.7; H 6.5; N 9.8. Found C 64.2; H 6.5; N 9.9) was treated with HCl/AcOH 1.3*N* to give the hydrochloride XII[4] (100% yield, $E_{1,9}$ = 0.60 Leu).

CBO-prolyl-prolyl-glycine (XIII)[7] (m.p. 103–108° ex AcOEt/pet. ether, $[\alpha]_D^{20}$ −80°, c 1, DMF. *Anal.* Calcd. for $C_{20}H_{25}N_3O_6 \cdot {}^1/_2H_2O$: C 58.2; H 6.4; N 10.2. Found C 58.2; H 6.4; N 10.1) was treated with HBr/AcOH to give the hydrobromide XIV which was condensed, without further purification, with *p*-nitrophenyl N^{α}-CBO-$N^{\omega,\omega}$-*bis*-CBO-argininate[8], in DMF with 2 equivalents of TEA, to give N^{α}-CBO-$N^{\omega,\omega}$-*bis*-CBO-arginyl-prolyl-prolyl-glycine[9] (XV) (60% yield; amorphous; $[\alpha]_D^{20}$ −50°, c 1, DMF. *Anal.* Calcd. for $C_{42}H_{49}N_7O_{11} \cdot {}^1/_2H_2O$: C 60.3; H 6.0; N 11.7; O 22.0. Found C 60.6; H 6.1; N 11.4; O 22.1) that was condensed in DMF, with DCCI, with phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester hydrochloride (XII) to give N^{α}-CBO-$N^{\omega,\omega}$-*bis*-CBO-arginyl-prolyl-prolyl-glycyl-phenylalanyl-O-acetyl-seryl-prolyl-phenylalanyl-$N^{\omega,\omega}$-*bis*-CBO-arginyl-isoleucyl-tyrosine benzylester (XVI)[10] (41% yield; m.p. 125°; $[\alpha]_D^{20}$ −35°, c 1, DMF. *Anal.* Calcd. for $C_{114}H_{131}N_{17}O_{25}$: C 64.0; H 6.2; N 11.1. Found C 63.6; H 6.2; N 11.0).

The protected hendecapeptide XVI was treated overnight in pyridine with 10 equivalents of SO_3-pyridine complex; dilution with water and extraction with dichloromethane afforded the O-tyrosyl sulphate XVII (90% yield; m.p. 130–135°; $[\alpha]_D^{20}$ −18°, c 1, DMF. *Anal.* Calcd. for $C_{114}H_{131}N_{17}O_{28}S$: C 61.7; H 6.0; N 10.7; S 1.4. Found C 61.8; H 6.3; N 10.4; S 1.9) which was hydrogenated in MeOH 80% (Pd/C) at 5 atm pressure: the solution was next treated with NaOH *N*/10 until a permanent alkaline reaction was achieved. The solvent was evaporated and the residue purified by counter-current distribution in a BuOH/EtOH/AcOH/H_2O (5:1:1:8) system to give XVIII (50% yield; m.p. 240° dec.; $[\alpha]_D^{20}$ −63°, c 0.9, AcOH 95%; $E_{1.9}$ = 0.39 Arg; 0.82 Glu; $E^a_{1.9}$ = 0.58 Arg; 1.23 Glu. *Anal.* Calcd. for $C_{65}H_{93}N_{17}O_{17}S \cdot 2CH_3COOH$: C 53.9; H 6.6; N 15.5. Found C 53.8; H 6.5; N 15.6) which was found homogeneous and showed amino acid composition, behaviour towards tripsin and chimotripsin[11] and the same biological properties[12] as natural phyllokinin, thus confirming the formula deduced from degradative experiments[13].

Riassunto. Viene riportata la sintesi della arginil-prolil-prolil-glicil-fenilalanil-seril-prolil-fenilalanil-arginil-isoleucil-tirosina-O-solfato, un polipeptide identico per proprietà chimiche, fisiche e biologiche alla phyllochinina, analogo naturale della bradichinina.

L. Bernardi, G. Bosisio,
R. de Castiglione, and O. Goffredo

Istituto Ricerche Farmitalia, Milano (Italy), April 12, 1966.

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[6] This protected peptide can be easily purified by chromatography on silica gel (eluent, top layer of the mixture benzene/AcOEt/AcOH/H_2O 10:10:2:1).
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[9] The peptide was purified by chromatography on disactivated silica gel (15% H_2O) (eluent, $CHCl_3$/AcOH 50:1).
[10] The peptide was purified by chromatography on silica gel (eluent, the same as [6]).
[11] We are indebted to Dr. A. Anastasi for these assays.
[12] We are indebted to Prof. V. Erspamer for these assays.
[13] We wish to express our thanks to Dr. B. Camerino, Director of this Research Institute, for his sustained interest in this work.

Biochemical, Genetical Studies on Host-Parasite Relationship: Variation in Fusaric Acid Production with Different Carbon Sources[1]

Fusarium vasinfectum Atk., the causal agent of cotton wilt has been shown to utilize macromolecules as the sole carbon source with the help of adaptive hydrolytic enzymes, the production of which increases in the presence of the respective substrates or host tissue extracts[2]. Hence it was of interest to study how the various carbon sources affect the production of the wilt toxin fusaric acid by this pathogen; our results are presented in the present communication.

A pathogenic isolate of *Fusarium vasinfectum* Atk. was grown in Richards medium with various amendments as described elsewhere[2]. Krebs cycle intermediates were amended as follows: 0.30*M* citric acid, 0.42*M* succinic acid and 0.42*M* fumaric acid were dissolved in Richards medium without glucose, to supply 2% carbon equivalent as contained in 5% glucose present in Richards medium and adjusting the pH to 5.5 ± 0.2. 15-day-old culture filtrates were adjusted to pH 4.0, and extracted thrice with equal volumes of ethyl acetate[3]. The solvent was

[1] This research has been financed in part by a grant made by the United States Department of Agriculture under No. P.L. 480. The authors wish to thank Prof. Dr. H. Kern of Switzerland for a generous gift of fusaric acid.
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