to benzilic acid rearrangement and of the 23,24 bond of similar compounds to retro aldol cleavage.[9] In a parallel study, we recently found the alkaline solvolysis of the ester in a ketol acetate to be facilitated by the adjacent carbonyl group or its hemiketal adduct.[10] Accordingly, treatment of datiscacin (**1**) with sodium carbonate in aqueous methanol for 12 hr at room temperature effected a smooth solvolysis of the 20-acetate ester group, to yield cucurbitacin I (**4**). The interrelation completed the proof of the structure of datiscacin (**1**), the first recognized cucurbitacin 20-acetate ester derivative.

Experimental Section

Melting points were determined on a Mettler FP2 melting point apparatus. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Ultraviolet spectra were recorded on a Coleman Hitachi EPS-3T recording spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian HA-100 spectrometer using TMS as an internal reference. Mass spectra were recorded on either Hitachi Perkin-Elmer RMU-63 or AEI MS-902 spectrometers, equipped with direct insertion probes. High-pressure liquid chromatography was carried out on a Waters ALC-202/401 liquid chromatographic system. Analytical and preparative tlc were carried out on Brinkmann Silplates. Petroleum ether refers to the fraction of bp 60–68°. Evaporations were carried out at reduced pressure below 40°. Analyses were carried out by Spang Microanalytical Laboratories, Ann Arbor, Mich.

Extraction and Fractionation.—The dried ground roots of *Datisca glomerata* Baill. (10 kg)[11] were continuously extracted with chloroform for 20 hr. Evaporation gave crude extract A (270 g), which was partitioned between water (500 ml) and chloroform (two 1-l. portions). The chloroform solution was evaporated to give a viscous brown residue (C, 260 g). The aqueous solution was freeze-dried to yield fraction B (3 g). Fraction C was partitioned between aqueous methanol (1:9, 1 l.) and petroleum ether (three 1-l. portions). Evaporation of the aqueous methanol solution gave fraction D (230 g) and the combined petroleum ether extracts yielded residue E (25 g). Fraction D was partitioned between aqueous methanol (2:8, 1 l.) and carbon tetrachloride (two 1-l. portions). The aqueous methanol layer gave fraction F (154 g) and the carbon tetrachloride layer gave fraction G (70 g).

Isolation of Datiscacin.—Fraction G (70 g) was further fractionated by column chromatography on silica gel (1.2 kg, 70–325 mesh). Elution with chloroform followed by 5% methanol–chloroform yielded a fraction (H, 1.35 g) enriched in datiscacin. Fraction H was separated by repeated preparative tlc with diethyl ether and the main band was eluted with methanol to give a light-yellow gum. The gum was crystallized from ethanol to give datiscacin (**1**, 25 mg), $C_{32}H_{44}O_8$: mp 208–212°; $[\alpha]^{23}D$ $-18°$ (c 0.87, $CHCl_3$); uv $\lambda_{max}^{CHCl_3}$ 231 nm (ϵ 9600), 268 (5400); ir (KBr) 2.79–2.88, 3.35, 3.41, 5.81, 5.95, 6.13, 7.14, 7.30, 7.89, 8.81, 8.89, 9.02, 10.1, and 12.6 μ; nmr ($CDCl_3$) τ 3.17 (1 H, d, J = 16 Hz), 3.75 (1 H, d, J = 16 Hz), 4.19 (1 H, s), 4.29 (1 H, d, J = 2.5 Hz), 4.47 (1 H, m), 5.90 (2 H, m), 8.19 (3 H, s), 8.62 (3 H, s), 8.63 (3 H, s), 8.74 (3 H, s), 8.76 (3 H, s), 8.82 (3 H, s), 8.92 (3 H, s), 9.12 (3 H, s), and 9.17 (3 H, s); mass spectrum m/e 496, 478, 401, 385, 383, 369, 367, 219, 164, 113, 96, and 43.

Anal. Calcd for $C_{32}H_{44}O_8 \cdot {}^1/_2H_2O$: C, 67.94; H, 8.02. Found: C, 67.81; H, 8.18.

Acetylation of Datiscacin (1) to Triacetate 2.—A solution of datiscacin (**1**, 10 mg) in anhydrous pyridine (0.5 ml) and acetic anhydride (0.5 ml) was stirred overnight at room temperature under nitrogen. The solution was evaporated *in vacuo* and the residue was dissolved in ethanol and reevaporated. The oily residue (10 mg) was separated by preparative tlc with diethyl ether. The product (8 mg) was crystallized from diethyl ether–hexane to give **2** (5 mg): mp 119–120°; $[\alpha]^{23}D$ $-43°$ (c 1.40, $CHCl_3$); ir (KBr) 2.80, 3.35–3.52, 5.75, 5.92, 6.15, 6.90, 7.30, 8.00, 8.30, 9.65, and 13.4 μ; nmr ($CDCl_3$) τ 2.82 (1 H, d, J = 16 Hz), 3.58 (1 H, d, J = 16 Hz), 4.16 (1 H, m), 4.78 (1 H, m), 5.06 (1 H, s), 7.76 (3 H, s), 7.92 (3 H, s), 8.08 (3 H, s), 8.36 (6 H, s), 8.48 (6 H, s), 8.60 (6 H, s), and 8.88 (6 H, s); mass spectrum m/e 580, 538, 487, 485, 411, 409, 367, 351, 309, 111, 96, 79, 60, 45, and 43.

Anal. Calcd for $C_{36}H_{48}O_{10}$: C, 67.48; H, 7.55. Found: C, 67.10; H, 7.72.

Periodic Acid Titrations.—The titrations were performed essentially according to the procedure of Jackson.[12] A solution of substrate (13 mg) in 95% ethanol (3.00 ml) was treated with 0.043 M periodic acid (2.00 ml) in an erlenmeyer flask (25 ml). The flask was kept in the dark under nitrogen for 7 days. The solution was then treated with 0.056 M iodine solution (7.02 ml) and titrated with sodium arsenite (0.10 M, 3.06 ml) to the blue starch end point. Datiscacin diacetate (**2**) consumed no periodic acid and was recovered unchanged. Cucurbitacin I diacetate (**5**) consumed 1.1 molar equiv of periodic acid.[8]

Solvolysis of Datiscacin (1) to Cucurbitacin I (4).—A solution of datiscacin (**1**, 10 mg) in methanol (2 ml) was treated with aqueous sodium carbonate (0.1 M, 0.5 ml) and allowed to stand overnight at room temperature. The mixture was neutralized with acetic acid and extracted with ethyl acetate. Evaporation of the ethyl acetate solution gave a residue (8 mg) which was separated by preparative tlc with 7% methanol–chloroform. Elution of the major band followed by evaporation gave a crude product (4.5 mg) which was further separated by high-pressure liquid chromatography [column, Corasil II,[13] 3 ft × 0.375 in.; solvent, hexane–ether (3:7)]. The crystalline product (0.9 mg, from ether–petroleum ether) was characterized as cucurbitacin I (**4**) by mixture melting point, mass spectrum, tlc, and high pressure lc comparisons with an authentic sample.

Registry No.—**1**, 38308-89-3; **2**, 38308-90-6; **4**, 2222-07-3.

(9) *Cf.* D. Lavie, Y. Shvo, O. R. Gottlieb, and E. Glotter, *J. Org. Chem.*, **27**, 4546 (1962).

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(13) From Waters Associates Inc., Framingham, Mass.

A Synthesis of Homoserine Phosphate and a Blocked Derivative Suitable for Peptide Synthesis

THEODORE E. FICKEL AND CHARLES GILVARG*

Department of Biochemical Sciences, Princeton, University, Frick Chemical Laboratory, Princeton, New Jersey 08540

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In the course of our studies of oligopeptide transport in *E. coli* it became of interest to synthesize peptides containing the amino acid homoserine phosphate. A search of the literature revealed no suitable chemical synthesis for either homoserine phosphate or a blocked derivative thereof. Homoserine phosphate has been prepared enzymically with crude yeast homoserine kinase;[1] we found the method cumbersome and not appropriate for the production of the relatively large quantities of blocked derivatives required for peptide synthesis. We wish to report a simple synthesis leading to *O*-diphenylphosphorohomoserine benzyl ester tosylate in an overall yield of 17% starting with homoserine. The compound can either be introduced at the carboxyl end of a suitably blocked peptide or subjected to hydrogenolysis to yield homoserine phosphate in roughly 100% yield. The synthesis has been carried out starting with DL-homoserine and L-homoserine;

(1) Y. Watanabe and K. Shimura, *J. Biochem.*, **43**, 283 (1956).

during the process of converting L-homoserine to homoserine phosphate, 19% racemization was found to occur.

Both homoserine and homoserine phosphate are intermediates in the biosynthesis of threonine. Watanabe, Konishi, and Shimura[2,3] have characterized two enzymic reactions in yeast; the first converts homoserine to homoserine phosphate, and the second converts this intermediate to threonine. The same pathway has been shown to exist in *N. crassa*[4,5] and in *E. coli*.[6] Neither compound has been found to be a constituent of proteins. The tendency of a carboxyl activated homoserine derivative (such as homoseryl tRNA) to lactonize may well be the reason nature has not made use of homoserine as a protein constituent.

The obstacle to a successful synthesis of homoserine phosphate has been the tendency of homoserine[7] or any of its N-blocked derivatives to undergo lactonization in acidic medium.[2] Moreover, *N*-benzyloxycarbonylhomoserine methyl ester is extremely base sensitive; merely washing an ethereal solution of the ester with saturated sodium bicarbonate leads to quantitative conversion of the material to the corresponding lactone. Thus, neither acidic nor basic conditions can be employed to synthesize an ester suitable for phosphorylation. It is not possible to phosphorylate a derivative blocked only at the amino function owing to rapid, initial formation of a mixed acyl phosphate anhydride followed by lactonization. Other active esters would also be expected to lactonize; in fact, we found that *N*-benzyloxycarbonylhomoserine azide lactonizes as rapidly as it is formed. It is worthy of note that several syntheses exist for *O*-phosphate esters of threonine and serine;[8] the well-known resistance of these compounds to β-lactone formation explains the ready accessibility of these two β-*O*-phosphate esters.

The obvious choice for an esterification under neutral conditions is a carbenoid-type reaction utilizing either diphenyldiazomethane or diazomethane. Although we were not able to make a benzhydryl ester following the procedure of Hardegger, *et al.*,[9] we did successfully synthesize *N*-benzyloxycarbonylhomoserine methyl ester. The ester can be phosphorylated with diphenyl phosphochloridate; however, the *N*-benzyloxycarbonyl-*O*-diphenylphosphorohomoserine methyl ester obtained from this reaction is completely refractile to hydrazinolysis, presumably owing to steric hindrance. Steric hindrance has been proposed as the explanation for the marked resistance of *N*-trityl amino acid esters to hydrazinolysis and base-catalyzed hydrolysis.[10] Although one presumably could hydrolyze the ester with methanolic potassium hydroxide, the likelihood of racemization in such a procedure led us to search for a different synthesis.

Given these problems, we turned to the synthesis of an N-blocked homoserine benzyl ester using an esterification procedure which, to our knowledge, has not previously been used in peptide chemistry. The key step in our sequence is the esterification of *tert*-butoxycarbonylhomoserine with 1-benzyl-3-*p*-tolyltriazene in ether.[11] A disadvantage of the reaction is that it produces *p*-toluidine as a by-product; as this base accumulates during the course of the reaction, the ethereal solution of *tert*-butoxycarbonylhomoserine benzyl ester becomes sufficiently basic such that the benzyl ester which has formed is subject to base-catalyzed lactonization. We could find no solution to this dilemma and this reaction is responsible for the greatest loss in yield. The ester is phosphorylated with diphenyl phosphorochloridate[12] and the *tert*-butoxycarbonyl function is removed with boron trifluoride etherate in ether[13] to give *O*-diphenylphosphorohomoserine benzyl ester, which is crystallized as its tosic acid salt. This compound has been successfully used in several peptide syntheses which will be reported separately. It has also been deblocked to give pure homoserine phosphate, which is chromatographically and biologically identical with material prepared enzymically by the procedure of Watanabe and Shimura.[1]

Using a biological assay, we find that the homoserine phosphate produced by deblocking *O*-diphenylphosphoro-L-homoserine benzyl ester is 19% racemic. The particular difficulties in synthesizing a carboxyl-blocked derivative of homoserine have forced us to make use of an esterification procedure which, to our knowledge, has not been used to esterify optically active compounds. Since only in the esterification step is a homoserine ester derivative subjected to basic conditions, we ascribe this racemization to the action of the *p*-toluidine produced in that reaction as a by-product.

Experimental Section[14]

***N-tert*-Butoxycarbonyl-DL-homoserine.**—For the synthesis of this compound the DMSO method[15] proved to be the easiest. A heterogeneous solution of 2.0 g of DL-homoserine, 4.8 ml of triethylamine, and 2.6 ml of *tert*-butoxycarbonyl azide was stirred for 20 hr, after which time the solution was homogeneous. A noncrystallizable oil (4 g) was isolated which has a slight odor of DMSO. By the criterion of tlc, the material was homogeneous.

***N-tert*-Butoxycarbonyl-DL-homoserine Benzyl Ester.**—The acid (8.5 g) was dissolved in 200 ml of anhydrous ether. 1-Benzyl-3-*p*-tolytriazene (11.3 g) (previously recrystallized from hexane) in 50 ml of anhydrous ether was added over a period of 15 min to the stirred solution of *tert*-butoxycarbonylhomoserine. The reaction was allowed to proceed for 1.5 hr at room temperature; although the reaction was not complete by this time, it was terminated since lactonization began to occur (tlc). Remaining *tert*-butoxycarbonylhomoserine can be removed from the ether by aqueous extraction whereas the lactone cannot. The benzyl ester (8 g) (as an oil) was isolated which was free of lactone and free acid (tlc).

***O*-Diphenylphosphoro-DL-homoserine Benzyl Ester Tosylate.**—The benzyl ester (2.8 g) was phosphorylated with diphenyl

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(13) E. Schnabel, H. Klostermeyer, and H. Berndt, *Justus Liebigs Ann. Chem.*, **749**, 90 (1971).

(14) Tlc was performed on silica gel plates (Mann Research Laboratories) with 100% ethyl acetate as the developing solvent. Melting points are uncorrected.

(15) J. M. Stewart and J. D. Young, "Solid State Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969, p 28.

phosphorochloridate (2.5 ml) in CCl_4 and anhydrous pyridine; 3.8 g of an orange oil was isolated. The free amine, obtained by removal of the *tert*-butoxycarbonyl function with boron trifluoride, was precipitated from ether with an ethereal solution of tosic acid. The crude tosic acid salt (2 g) was filtered from the ether after standing overnight in the cold. The yield for each step was difficult to calculate owing to the fact that none of the intermediates could be crystallized. However, starting with 3.5 g (29 mmol) of DL-homoserine, 4.5 g (7.3 mmol) of the crude tosic acid salt was isolated which represents an overall yield of 25%. The crude tosic acid salt can be twice crystallized from alcohol and ether with a 70% recovery to give crystals with a melting point of 110–112°.

Anal. Calcd for $C_{30}O_9NSPH_{32}$: C, 58.7; N, 2.29; H, 5.23. Found: C, 59.20; N, 2.39; H, 5.33.

Conversion of L-homoserine to the corresponding tosic acid salt (mp 109–110°) was effected in a similar yield.

L-Homoserine Phosphate.—*O*-Diphenylphosphoro-L-homoserine benzyl ester tosylate (122 mg) was converted to the free amine and dissolved in 2 ml of distilled acetic acid; 100 mg of PtO_2/C was added and the reaction mixture was subjected to hydrogenolysis at room temperature and pressure. The progress of the reaction was monitored by assaying the phosphate content of an aliquot of the reaction mixture[16] treated with alkaline phosphatase; it was found that the reaction required 4 days to go to completion. Homoserine phosphate was isolated in quantitative yield; the material cochromatographs with homoserine phosphate prepared enzymically, is ninhydrin and phosphate positive,[17] and, after alkaline phosphatase treatment, cochromatographs with homoserine. In both cases one-dimensional chromatography was performed with Whatman Chromatography Paper No. 1 with phenol–water (80:20) as the developing solvent.[1]

Racemization Assay.—In order to determine the degree of racemization accompanying our synthesis of L-homoserine phosphate we made use of the auxotroph, *E. coli* M-145.[18] This organism can utilize L-homoserine in the place of three of its required amino acids, methionine, threonine, and isoleucine. It cannot, however, utilize homoserine phosphate as such owing to the impermeability of this anion. Thus, in order to assay the material for its optical purity, it was dephosphorylated with alkaline phosphatase.[19] The enzymic reaction produced homoserine in virtually quantitative yield (paper chromatography); any remaining homoserine phosphate will not interfere with the biological assay, as it cannot be utilized by the bacterium. As shown in Table I, the homoserine from L-homoserine phosphate is 81% as effective as an L-homoserine standard in supporting growth of the auxotroph, indicating that 19% of the synthetic material is D-homoserine phosphate.

TABLE I
GROWTH YIELD OF M-145 ON HOMOSERINE AND HOMOSERINE PHOSPHATE

Sample	Klett units per micromoles of material
L-Homoserine	530
DL-Homoserine	270
L-Homoserine phosphate[a]	430
DL-Homoserine phosphate[a]	270

[a] The number of micromoles of phosphate released by the alkaline phosphatase is taken to be the number of micromoles of homoserine available to the organism to support its growth. See Experimental Section for details.

Registry No.—*N-tert*-Butoxycarbonyl-DL-homoserine, 38308-92-8; DL-homoserine, 1927-25-9; triethylamine, 121-44-8; *tert*-butoxycarbonyl azide, 1070-19-5; *N-tert*-butoxycarbonyl-DL-homoserine benzyl ester, 38308-93-9; 1-benzyl-3-*p*-tolyltriazene, 17683-09-9; *O*-diphenylphosphoro-DL-homoserine benzyl ester tosylate, 38308-95-1; *O*-diphenylphosphoro-DL-homoserine benzyl ester, 38308-96-2; diphenylphosphorochloridate, 2524-64-3; L-homoserine phosphate, 4210-66-6; *O*-diphenylphosphoro-L-homoserine benzyl ester tosylate, 38308-98-4.

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Base-Catalyzed Condensation of Aldehydes with Ethyl Bis(diethylphosphonomethyl)phosphinate[1]

W. FRANKLIN GILMORE* AND JOSEPH W. HUBER, III

Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, Mississippi 38677

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As a possible synthesis of ethyl (diethylphosphonomethyl)vinylphosphinates **4** we have explored the base-catalyzed condensation of aldehydes with ethyl bis(diethylphosphonomethyl)phosphinate (**2**). Based on previously reported results[2] as well as our experience, the base-catalyzed condensation of tetraethyl methylenediphosphonate (**1**) with aldehydes is an excellent synthetic method for vinylphosphonates. We therefore expected that, during the course of the base-catalyzed reaction of **2** with aldehydes, diethyl phosphate ion would be eliminated with the formation of **4**. Instead, **5** was eliminated with the formation of **3**. The course of the reaction was the same when a number of solvents (benzene, ethanol, DMSO, ether, 1,2-dimethoxyethane) and a number of bases (sodium hydride, sodium ethoxide, potassium *tert*-butoxide) were used. The reaction is stereoselective with formation of predominantly the *trans*-vinylphosphonates. The stereochemistry was assigned on the basis of the nmr spectra and gas chromatograms.[3]

In order to change the electronic and steric properties of the central phosphorus atom, phenyl bis(diethylphosphonomethyl)phosphinate and isopropyl bis(diisopropylphosphonomethyl)phosphinate were prepared and reacted with isobutyraldehyde. The results were the same as with **2**. No attempt has been made to maximize these factors. From our very limited study we cannot indicate why the C–P bond of a phosphinate is cleaved in preference to a C–P bond of a phosphonate. Examination of models of possible transition states and intermediates has not lead us to an explanation.

One practical utilization of this reaction is the synthesis of compounds such as **5**. Such compounds are

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