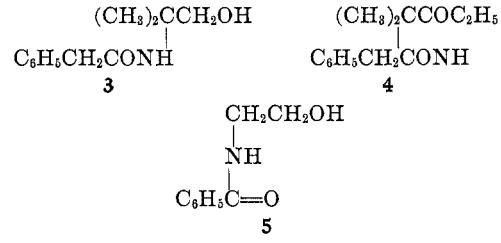
When **1** was treated with an excess of sodium borohydride, a compound **3** was obtained. Any doubt about the proposed structure for **3** was eliminated when acid hydrolysis of **3** afforded phenylacetic acid and the sulfate ester of 2-amino-2-methyl-1-propanol.[3] Compound **3** reacted with phenyl isocyanate to yield the corresponding carbamate. Spectral data also confirmed this structure.



3 **4**

5

The reduction of **1** with a stoichiometric quantity of reagent gave a mixture of starting material **1**, the alcohol **3**, and the ethyl ester **4**. This compound, **4**, was separated from **1** and **3** by chromatographing over acidic alumina. The same compound was obtained by esterification of α-methyl-N-phenylacetyl-α-alanine with ethanol and sulfuric acid.

The reduction of 2-phenyl-2-oxazoline-5-one (**2**), with an excess sodium borohydride resulted in a 90% yield of N-(2-hydroxyethyl)benzamide (**5**) as a thick liquid. This alcohol was identified by its reaction with phenyl isocyanate to form the known phenyl carbamate.[4]

Experimental Section

All melting points were taken with a Hoover–Johns melting point apparatus and are uncorrected; analyses were carried out by Mr. Ed Hoff. Nmr spectra were determined in $CDCl_3$ with TMS as an internal standard, using a Varian A-60 spectrometer. The infrared spectra wre obtained from potassium bromide disks on a Perkin–Elmer Model 237 spectrophotometer.

Sodium Borohydride Reduction of the Aza Lactone[5] 1.—The aza lactone **1** (500 mg) was dissolved in a mixture of tetrahydrofuran, ethanol, and water (15 ml, 1:1:1), sodium borohydride (50 mg) was added in small portions with stirring, and the reaction mixture was kept at room temperature for 20 hr. When the reaction mixture was worked up, a thick liquid (500 mg) was obtained. On chilling in a Dry Ice–acetone bath it solidified and was crystallized from ether in shining colorless cubes, mp 75–76° (470 mg, 91%); ν_{max} 3370 cm^{-1} (s), 3240 (s), 1645 (s), 1600 (m), 1580 (s), and 1080 (s). The nmr spectrum showed a sharp peak at δ 7.30 (5 H, phenyl), a broad peak between 5.65 and 5.90 (1 H, NH), a broad peak between 4.5 and 4.75, centered at 4.62 (1 H, OH), one sharp peak at 3.5 (4 H), and a sharp peak at 1.22 (6 H, methyls).

Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.62; H, 8.25; N, 6.77. Found: C, 69.66; H, 8.25; N, 6.55.

Reaction of **3** with phenyl isocyanate gave the expected phenyl carbamate as a white solid, which was crystallized from acetone in shining white needles, mp 157°.

Anal. Calcd for $C_{19}H_{22}N_2O_3$: C, 70.00; H, 6.80; N, 8.59. Found: C, 69.85; H, 6.67; N, 8.64.

When the same reaction was carried out with a stoichiometric quantity of sodium borohydride under the same experimental conditions a mobile liquid was obtained. The crude liquid in the infrared showed the presence of some unreacted aza lactone (ν_{max} 1805 cm^{-1}), hydroxy (ν_{max} 3400 cm^{-1}), and ester (ν_{max} 1725 and 1180 cm^{-1}). On chromatography over acidic alumina with petroleum ether–ether as the eluting agent (4:1), a white solid was obtained in 31% yield, mp 101°, which was crystallized from a petroleum ether–ether mixture in fine silky needles: mp 101°, ν_{max} 3230 (s) 1725 (s), 1640 (s), 1602 (w), 1565 (s), and 1180 cm^{-1} (s). The nmr spectrum showed a sharp peak at δ 7.31 (5 H, phenyl), a broad peak between 6.25 and 6.10 (1 H, NH), a quartet between 4.30 and 3.95 (J = 7 cps, 2 H, OCH_2CH_3), one sharp peak at 3.5 (2 H, $CH_2C_6H_5$), one sharp peak at 1.5 (6 H, methyls), and a triplet between 1.34 and 1.11 (J = 7 cps, 3 H, CH_2CH_3).

Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.53; H, 7.69; N, 5.63. Found: C, 67.56; H, 7.68; N, 5.48.

The same compound was obtained by esterification of α-methyl-N-phenylacetyl-α-alanine with ethanol and sulfuric acid, mp 101°, alone or mixed with the above compound.

The ether eluent afforded colorless solid alcohol **3** in 60% yield, mp 75–76°, alone or mixed with the known sample of the alcohol.

Hydrolysis of 3 with Sulfuric Acid.—The compound **3**, mp 75–76° (400 mg), was hydrolyzed with sulfuric acid (15 ml, 30%) for 4 hr. A white solid (270 mg) was obtained, which was crystallized from petroleum ether in shining white flakes, and was confirmed to be phenylacetic acid (yield 100%), mp 78–79°, alone or mixed with the authentic sample of phenylacetic acid.

The aqueous sulfuric acid solution was then neutralized with barium hydroxide solution, and the precipitated barium sulfate was filtered off. The filtrate was evaporated to dryness on a steam bath. A brown gummy material was left. On trituration with a few drops of methanol, a white amorphous solid (about 50 mg) was separated, which on crystallization from methanol melted at 260–262° (with vigorous evolution of gas). Buckles and Mock[3] reported the melting point of the sulfate ester of 2-amino-2-methyl-propanol as 253–255° dec.

Sodium Borohydride Reduction of the Aza Lactone[6] 2.—The azalactone **2** (1.6 g) was reduced with sodium borohydride (190 mg) in a solution of tetrahydrofuran, ethanol, and water (30 ml, 1:1:1). The thick liquid (1.4 g; 88%) had ν_{max} 3380 (s), 3220 (hump), 1645 (s), 1600 (m), 1565 (s), and 1090 cm^{-1} (s). Reaction of this compound with phenyl isocyanate gave the known phenyl carbamate which was crystallized from acetone in white flakes, mp 197°.[4]

Registry No.—Sodium borohydride, 1303-74-8; **1**, 22929-09-5; **2**, 1199-01-5; **3**, 1569-06-8; **3** phenyl isocyanate, 22929-14-2; **4**, 29292-12-0; **5**, 18838-10-3.

(3) R. E. Buckles and G. V. Mock, *J. Amer. Chem. Soc.*, **70**, 1275 (1948).

(4) O. Jeger, J. Norymberski, S. Szpilfogel, and V. Prelog, *Helv. Chim. Acta*, **29**, 684 (1946); *Chem. Abstr.*, **40**, 46567 (1946).

(5) S. W. Cornforth, "Chemistry of Penicillin," H. T. Clarke, *et al.*, Ed., Princeton University Press, Princeton, N. J., 1949, pp 688–848; *Chem. Abstr.*, **49**, 3141*a* (1955).

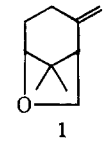
Total Synthesis of (±)-Karahana Ether

ROBERT M. COATES AND LAWRENCE S. MELVIN, JR.[1]

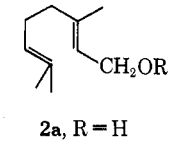
Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois 61801

Received August 13, 1969

Three new monoterpenes have recently been isolated from Japanese hop, "Shinshu-wase."[2] One of the two ether components is karahana ether, to which structure **1** was assigned on the basis of chemical and spectroscopic evidence. We report here a brief total synthesis of (±)-karahana ether from geraniol (**2a**) which confirms this structural assignment.[3]



1



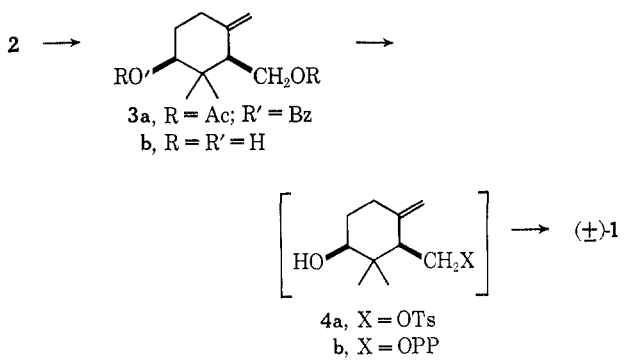
2a, R = H
b, R = Ac

(1) National Science Foundation Undergraduate Research Participant, Summer 1968.

(2) Y. Naya and M. Kotake, *Tetrahedron Lett.*, 1645 (1968).

(3) Another component, karahanaenone, has recently been synthesized: E. Demole and P. Enggist, *Chem. Commun.*, 264 (1969).

Geranyl acetate (**2b**) was treated with benzoyl peroxide, cupric benzoate, and cuprous chloride in acetonitrile at 70° as described by Breslow, Groves, and Olin.[4,5] The resulting mixture of benzoyloxy products containing the desired *cis* cyclic diester **3a** was saponified to the corresponding diols.[4b] Two careful chromatographies were necessary in order to obtain pure *cis*-diol (**3b**), the spectral properties of which are in agreement with the reported values.[4b]



3a, R = Ac; R′ = Bz
b, R = R′ = H

4a, X = OTs
b, X = OPP

Reaction of **3b** with 1 equiv of *p*-toluenesulfonyl chloride in pyridine at room temperature affords the cyclic ether, presumably by way of the less hindered primary tosylate **4a**.[6] The product of this reaction was identified as (±)-karahana ether by the complete coincidence of its richly detailed infrared and nmr spectra with the corresponding spectra of the natural product (kindly provided by Dr. Naya[2]).

The cyclization **4a** → **1** corresponds to the most probable biogenesis of karahana ether, namely, cyclization of the analogous monopyrophosphate (**4b**). Natural products based on structure **3**, but with the double bond located in the endocyclic positions, have recently been encountered, and probably have the same *cis* stereochemistry.[7,8] The facile conversion of **3b** into **1** also confirms the tentatively assigned *cis* configuration of **3**.[4b]

Experimental Section[9]

Geranyl Acetate (2b).—Commercial geraniol (Columbia Organic Chemicals Co., Inc.) contained substantial impurities and was therefore purified by conversion into the diphenylurethan. A solution of geraniol (63.2 g, 0.411 mol) and diphenylcarbamoyl chloride (95.0 g, 0.411 mol) in benzene (50 ml) was added cautiously to a suspension of 60% sodium hydride dispersion (16.4 g, 0.411 mol) in benzene (500 ml). The mixture was stirred at 90° for 1 hr under nitrogen. Water and ether were added successively, and the layers were separated. The aqueous layer was neutralized with 10% hydrochloric acid and extracted with additional ether. The combined organic extracts were washed with water and saturated sodium chloride, and then dried over sodium sulfate. The residue, after evaporation, was recrystallized from hexane, ether, and methanol to give geranyl diphenylurethan, yield 61 g (37%), mp 80.5–81° (lit.[10] mp 82°).

A solution of the urethan (56.9 g, 0.142 mol) and potassium hydroxide (17.9 g, 0.319 mol) in 95% ethanol (500 ml) was heated at reflux for 4 hr.[11] The cooled solution was diluted with water and the product was extracted into ether. The ether solution was dried (Na_2SO_4) and evaporated and the residual oil was distilled under reduced pressure to give geraniol **2a**: yield 16.8 g (77%), bp 66–67° (0.4 mm), 95+% purity by glpc (90-P3, 15% Carbowax 20M, 165°). Geraniol (16.1 g) was then converted into geranyl acetate (**2b**) by reaction with acetic anhydride and pyridine:[4b,12] yield 16.1 g (82%); bp 53° (0.1 mm), 99% pure by glpc (90-P3, 15% Carbowax 20M, 162°).

***cis*-2,2-Dimethyl-3-hydroxy-6-methylenecyclohexanemethanol (3b).**[4]—A solution of geranyl acetate (15.9 g, 86.3 mmol), benzoyl peroxide (10.5 g, 43.2 mmol), cupric benzoate (896 mg, 2.94 mmol), and cuprous chloride (134 mg, 1.35 mmol) in acetonitrile (45 ml) was heated at 70° for 17 hr.[4a,5] The cooled solution was diluted with 10% sodium carbonate and extracted with ether. The ether solution was washed with 10% sodium carbonate and water, dried (Na_2SO_4), and evaporated. Most of the excess geranyl acetate [7.02 g, bp 55–57° (0.15 mm)] was separated from the crude product by distillation, and then the pot residue (13.5 g) was chromatographed on a column of silica gel (540 g, H/D 7). Elution with 0–10% ether–pentane afforded another 0.71 g of geranyl acetate and a mixture of mainly benzoyl–acetyl diesters (7.7 g, 58%) consisting of *cis* diester **3a** (*ca.* 23%), *trans* diester (*ca.* 16%), and acyclic diester (*ca.* 51%)[4b] as estimated from nmr spectra and glpc analyses (90 P3, 20% SE-30, 215°). The nmr signals for the saturated methyl group of the two cyclic diesters (*cis*, τ 8.91, 8.99; *trans*, τ 8.88, 9.02) agree with the corresponding literature values (τ 8.90, 8.98; 8.88, 9.01).[4b]

Anal. Calcd for $C_{19}H_{24}O_4$ (mixture of diester isomers): C, 72.13; H, 7.65. Found: C, 72.07; H, 7.62.

A solution of the diester mixture (5.79 g, 18.9 mmol) and potassium hydroxide (4.23 g, 75.6 mmol) in 4:1 (v/v) methanol–dioxane (160 ml) was heated under reflux for 1 hr.[4b] The cooled reaction mixture was poured into water and extracted with ether. The diol mixture (3.21 g) obtained after drying (Na_2SO_4) and evaporating the ethereal solution was chromatographed on a silica gel column (120 g, H/D 6.2), eluting 20-ml fractions with 40% ether-ligroin. Fractions 10–14 were combined (567 mg) and a 475-mg portion of this partially purified *cis* diol was rechromatographed on 19 g of silica gel eluting with 10–50% ether–ligroin. The *cis* diol (**3b**) obtained (375 mg, 63% based on available *cis* diester) was pure according to glpc (Hy-Fi, 5% SE-30, 142°), tlc (75% EtOAc–$CHCl_3$), and nmr analysis: τ 5.10 and 5.27 (2 br s, 2 H, $=CH_2$), 6.12 and 6.36 (eight-line ABX, 2 H, J_{AB} = 11 Hz, J_{AX} = 7 Hz, J_{BX} = 3 Hz, CH_2OD), 6.58 (br t, 1 H, $J \cong 4$ Hz, CHOD), and 9.00 and 9.03 [2 s, 6 H, $C(CH_3)_2$] [lit.[4b] τ 5.05 and 5.25, 6.20 (m), 6.55, 9.00, and 9.05, respectively].

Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.55; H, 10.66. Found: C, 71.03; H, 10.39.

8,8-Dimethyl-2-methylene-6-oxabicyclo[3.2.1]octane [(±)-Karahana Ether, 1].—*p*-Toluenesulfonyl chloride (281 mg, 1.48 mmol) was added to a solution of the *cis*-diol **3b** (241 mg, 1.41 mmol) in pyridine (5 ml). After 45 min at room temperature, the solution was poured into water and extracted with petroleum ether (bp 30–60°). The petroleum ether extracts were washed with 10% hydrochloric acid and water, dried (Na_2SO_4), and evaporated. The residual yellow oil (170 mg) had a camphor-like smell and appeared to be essentially pure by tlc analysis (50% ether–petroleum ether). Chromatography on 6.8 g of silica gel and elution with petroleum ether afforded 63 mg (29%) of (±)-karahana ether (**1**) pure according to glpc (Hy-Fi, 5% SE-30, 91°) and nmr. The low recovery may be due to the volatility of **1** [bp 50–53° (15 mm)].[2] The infrared and nmr spectra of this material correspond in every detail with the spectra of natural karahana ether. In particular, the unusually rich fingerprint region (25 sharp bands at 700–1400 cm^{-1}) of the two infrared spectra matched precisely in both position and relative intensity.

(4) (a) R. Breslow, J. T. Groves, and S. S. Olin, *Tetrahedron Lett.*, 4717 (1966); (b) S. S. Olin, Ph.D. Thesis, Columbia University, 1967; *Dissertation Abstr.*, **28B**, 4947 (1968).

(5) J. K. Kochi, *J. Amer. Chem. Soc.*, **84**, 1572 (1962).

(6) For an example of selective primary over secondary tosylation, see J. A. Marshall and J. J. Partridge, *Tetrahedron*, **25**, 2159 (1969).

(7) F. Bohlmann, C. Zdero, and H. Kapteyn, *Justus Liebigs Ann. Chem.*, **717**, 186 (1968).

(8) R. M. Coates and L. S. Melvin, Jr., unpublished results.

(9) Melting points are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Model 137 infracord as thin films. Nmr spectra were obtained with Varian Associates spectrometers (Models A-60, A-60A, A-56/50) in chloroform-*d* with tetramethylsilane as internal standard. Glpc analysis were carried out with either a Wilkins Aerograph A90-P3 or Hy-Fi Model 600D as indicated. Microscope slides coated with silica gel were used for the tlc analyses with 5% phosphomolybdic acid in 95% ethanol as staining reagent.

(10) J. L. Simonsen, "The Terpenes," Vol. I, Cambridge University Press, London, 1931, p 33.

(11) R. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, **28**, 1086, (1963).

(12) J. Knight and E. S. Waight, *J. Chem. Soc.*, 2830 (1955).

Registry No.—1, 22922-43-6.

Acknowledgments.—We wish to thank Dr. Naya (Institute of Food Chemistry, Osaka, Japan) for the spectra of natural karahana ether and the National Institutes of Health and the National Science Foundation for partial support of this research.

A Modified Support for Solid-Phase Peptide Synthesis Which Permits the Synthesis of Protected Peptide Fragments[1]

DAVID L. MARSHALL

Department of Chemistry, Memphis State University, Memphis, Tennessee 38111

IRVIN E. LIENER

Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55101

Received August 18, 1969

Peptide products obtained by the solid-phase technique are normally not suited for use in subsequent coupling reactions owing to removal of N-terminal and side-chain protecting groups during the cleavage step. We have now developed a modified polymer support which retains the former advantages of the solid-phase technique but also permits removal of the peptide product with protecting groups intact.

The modified support is easily synthesized from the chloromethylated polystyrene resin (**1**) used in conventional solid-phase procedures by reaction with the O-carbonate ester of *p*-mercaptophenol[2] (**2**). The first N-protected amino acid is then coupled to the phenol–sulfide resin (**3**) by means of the mixed anhydride method or with dicyclohexylcarbodiimide. The remaining amino acids are introduced in the usual manner.[3] To remove the peptide from the support, the sulfide is oxidized to the sulfone (**4**) with hydrogen peroxide in acetic acid. This converts the anchoring ester linkage into an activated ester capable of acylating an amine. Thus the peptide, by acylating an amino acid (**5**), is released from the polymer support with the various side-chain and N-terminal protecting groups still in place and is lengthened by one amino acid at the C-terminal end (Scheme I).

The applicability of this modified support for the synthesis of peptides was first tested by the synthesis of N-benzoylglycine from a benzoylated phenol–sulfide polymer. Then additional peptides were prepared, including the sequence 180–184 of human growth hormone.[4] The model peptide, N-benzoyl-L-leucylglycine ethyl ester,[5] was prepared as a test for racemization which might occur during the acylation involving the active, insoluble ester. The optical rotation and melting point of the product prepared by this technique were in agreement with the values for the L-peptide indicating that little or no racemization had occurred.

The use of this type of convertible protecting group in conventional peptide synthesis was recently described by Johnson and Jacobs.[6] They report the peptide linkage to be stable during the H_2O_2 oxidation. However, cysteine, methionine, and tryptophan would be affected by this treatment. These amino acids could be incorporated by using them as the amino acid to be acylated by the activated, insoluble ester.

Experimental Section[7]

Preparation of Modified Polymer Support (3).—Four grams of chloromethylated polystyrene[8] was suspended in 25 ml of dimethylformamide (DMF) and refluxed for 3 hr with a methanol solution containing 1.2 g (6 mmol) of the O-carbonate ester of *p*-mercaptophenol (**2**) and 0.72 g (18 mmol) of NaOH. The resin was filtered and washed successively with DMF, methanol, acetic acid, 1 *N* HCl in acetic acid, and methanol and then dried. As judged by weight increase, the modified polymer contained *ca.* 0.91 mmol/g of phenol–sulfide groups.

N-Benzoylglycine.—The phenol–sulfide resin (**3**), suspended in DMF, was benzoylated with benzoyl chloride in the presence of pyridine to give a resin containing *ca.* 0.8 mmol/g of benzoyl groups as judged by weight increase. Oxidation with H_2O_2 in acetic acid at room temperature for 12 hr converted the benzoylated polymer into an active ester resin. Glycine (as the sodium salt) was added to a DMF–H_2O suspension of the resin and stirred for 24 hr, at which time a ninhydrin test on an aliquot indicated no free glycine. Filtration and acidification of the filtrate followed by evaporation of the DMF–H_2O gave a residue of N-benzoylglycine. Recrystallization from H_2O gave crystals, mp 189–190° (lit. mp 190°).

N-*p*-Nitrobenzyloxycarbonyl-L-leucyl-L-γ-benzylglutamylglycine.—In this procedure the first amino acid coupled to the polymer is the one which will be second from the C-terminal end in the final product. For this tripeptide, N-*t*-butyloxycarbonyl-L-glutamic acid γ-benzyl ester (0.53 g, 1.5 mmol) was dissolved in 15 ml of methylene chloride and added to 1 g of modified support (**3**) contained in a reaction vessel similar to that described by Merrifield.[3] Dicyclohexylcarbodiimide (0.31 g, 1.5 mmol) dissolved in 15 ml of methylene chloride was then added and stirred overnight with a mechanical stirring motor and rod at a rate just fast enough to keep the resin well suspended. The resin was filtered, washed with ethanol, acetic acid, and ethanol, and dried. The amount of glutamic acid coupled to the support was *ca.* 0.8 mmol/g. Following deprotection and neutralization of the glutamyl amino group, the next amino acid and N-terminal one for this product, N-*p*-nitrobenzyloxycarbonyl-L-leucine (0.52 g, 1.6 mmol), was added as a methylene chloride solution along with 0.33 g (1.6 mmol) of dicyclohexylcarbodiimide. The leucyl–glutamyl polymer was filtered and washed as before. The dipeptide polymer was treated with 2 ml of 30% H_2O_2 in 20 ml of acetic acid with stirring for 12 hr at room temperature. After filtering and washing with ethanol, removal of the peptide from the polymer was accomplished by stirring for 24 hr with 0.75 mmol of glycine (as the sodium salt) in a DMF–H_2O solvent. The mixture was filtered and the filtrate was evaporated to dryness. The residue was dissolved in water, the pH was adjusted to 3.5, and the precipitate which formed was collected by decantation. The wet residue was dissolved in absolute ethanol and evaporated to dryness. A white, granular product was obtained from this residue by precipitation from ethyl acetate–petroleum ether; this was followed by trituration of the precipitate with petroleum ether. The protected tripeptide amounted

(1) Supported in part by Grant GM-12837 from the United States Public Health Service to I. E. L.

(2) T. Zincke and C. Ebel, *Chem. Ber.*, **47**, 1100 (1915).

(3) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).

(4) C. H. Li, W. K. Liu, and J. S. Dixon, *J. Amer. Chem. Soc.*, **88**, 2050 (1966).

(5) N. A. Smart, G. T. Young, and M. W. Williams, *J. Chem. Soc.*, 3902 (1960).

(6) B. J. Johnson and P. M. Jacobs, *Chem. Commun.*, 73 (1968).

(7) Melting points were determined using Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Rudolph polarimeter, Model 80.

(8) Supplied by Bio-Rad as Bio-Beads SX-2, 1.5 mequiv of Cl/g.