(15) F. A. Bovey in "Chemistry and Biology of Peptides," J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 3.
(16) A. I. R. Brewster, V. J. Hruby, A. F. Spatola, and F. A. Bovey, *Biochemistry*, **12**, 1643 (1973).
(17) M. I. Blake, H. L. Crespi, V. Mohan, and J. J. Katz, *J. Pharm. Sci.*, **50**, 425 (1961).
(18) D. S. Berns, *J. Amer. Chem. Soc.*, **85**, 1676 (1963); D. S. Berns, *Biochemistry*, **2**, 1377 (1963), and related references.
(19) V. J. Hruby and A. F. Spatola, unpublished results.
(20) A. T. Blomquist, D. H. Rich, V. J. Hruby, L. L. Nangeroni, P. Glose, and V. du Vigneaud, *Proc. Nat. Acad. Sci. U. S.*, **61**, 688 (1968).
(21) A. T. Blomquist, D. H. Rich, B. A. Carlson, G. A. Allen, V. J. Hruby, H. Takashima, L. L. Nangeroni, P. Glose, and V. du Vigneaud, *Proc. Nat. Acad. Sci. U. S.*, **64**, 263 (1969).
(22) V. du Vigneaud, J. D. Meador, M. F. Ferger, G. A. Allen, and A. T. Blomquist, *Bioorg. Chem.*, **1**, 123 (1971).
(23) D. Yamashiro, *Nature (London)*, **201**, 76 (1964).
(24) D. Yamashiro, D. Gillessen, and V. du Vigneaud, *J. Amer. Chem. Soc.*, **88**, 1310 (1966).
(25) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).
(26) R. B. Merrifield, *Advan. Enzymol.*, **32**, 221 (1969).
(27) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 505 (1970).
(28) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(29) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Biol. Chem.*, **237**, 1563 (1962).
(30) D. Yamashiro, D. B. Hope, and V. du Vigneaud, *J. Amer. Chem. Soc.*, **90**, 3857 (1968).
(31) V. J. Hruby, L. E. Barstow, and T. Linhart, *Anal. Chem.*, **44**, 343 (1972).
(32) L. Corley, D. H. Sachs, and C. B. Anfinsen, *Biochem. Biophys. Res. Commun.*, **47**, 1353 (1972).
(33) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).
(34) R. B. Merrifield and M. A. Corigliano, *Biochem. Prep.*, **12**, 98 (1968).
(35) V. J. Hruby and L. E. Barstow, *Macromol. Syn.*, **4**, 91 (1972).
(36) G. Barany and R. B. Merrifield, *Cold Spring Harbor Symp. Quant. Biol.*, **37**, 121 (1973).
(37) L. T. Skeggs, K. E. Lentz, J. R. Kahn, F. E. Dorer, and M. Levine, *Circ. Res.*, **25**, 451 (1969).
(38) M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 5688 (1959); we have obtained similar yields in our laboratory.
(39) M. Manning, *J. Amer. Chem. Soc.*, **90**, 1348 (1968).
(40) The calculations for nonapeptide yields in solution syntheses are based on the limiting reagent, that is, the growing peptide chain, as reported in ref 38 (see pp 5690–5691; similar results were obtained in our laboratory), and *not* on the added active esters which are usually present in excess (10–40%). Intermediates are isolated. The overall yields for the nonapeptide by SPPS are based on the initial amino acid substitution (limiting reagent) on the chloromethylated resin, and *not* on protected amino acid used in each step which is usually added in two- to fourfold excess (see Experimental Section and Tables I and II for the actual amounts used in the syntheses reported here.)
(41) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(42) A. T. Blomquist, G. T. Taylor, D. A. Cornelius, G. A. Allen, D. H. Rich, and C. Clericuzio, *J. Org. Chem.*, in press.
(43) K. W. Ehler, Ph.D. Dissertation, University of Arizona, 1972.
(44) K. Esko, S. Karlsson, and J. Porath, *Acta Chem. Scand.*, **22**, 3342 (1968).
(45) Two different R_f values for the [1-hemi-D-cystine]oxytocin were quoted in that paper also, R_f 0.44 and 0.37.[24]
(46) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
(47) D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *J. Amer. Chem. Soc.*, **85**, 3686 (1963).
(48) M. Manning and V. du Vigneaud, *J. Amer. Chem. Soc.*, **87**, 3978 (1965).
(49) P. Holton, *Brit. J. Pharmacol. Chemother.*, **3**, 328 (1948).
(50) R. A. Munsick, *Endocrinology*, **66**, 451 (1960).
(51) C. G. VanDongen and R. L. Hays, *Endocrinology*, **78**, 1 (1966).
(52) M. E. Hadley and V. J. Hruby, manuscript in preparation.

A Synthesis of Racemic Ipomeamarone and Epiipomeamarone

Leo T. Burka*[1] and Benjamin J. Wilson

Center in Environmental Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

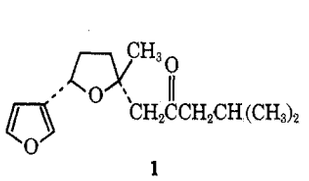
Thomas M. Harris

Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37232

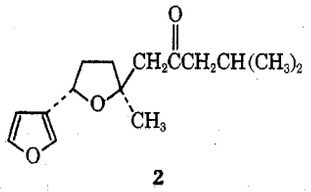
Received February 22, 1974

A synthesis of the sesquiterpene ipomeamarone (1) is described. Reaction of the anion of dimethyl 2-oxo-4-methylpentanephosphonate with 1-acetoxy-1-(3-furyl)-4-pentanone (6b) followed by hydrolysis produced 1 as well as epiipomeamarone (2). Kinetic and equilibrium mixtures from the cyclization reaction contained 1 and 2 in approximately equal amounts. The epimers were separated by high-pressure liquid chromatography and characterized.

The presence of the sesquiterpene ipomeamarone (1)[2] in mold-damaged sweet potatoes is well known.[3,4] The enantiomer of ipomeamarone and an epimer (2) have been found in *Myoporum deserti*.[5-7] As part of an investigation of other toxic metabolites found in moldy sweet potatoes, a convenient synthetic source of ipomeamarone was required.

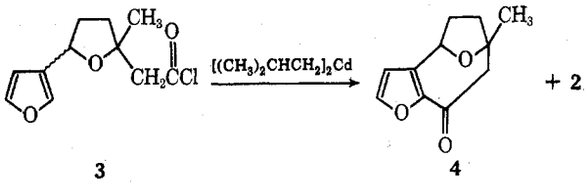


1



2

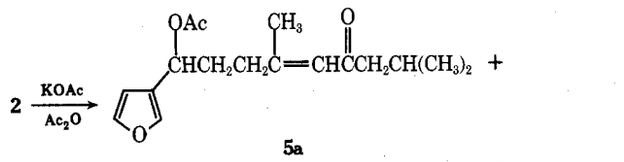
Kubota has described a synthesis in which a key step was addition of diisobutylcadmium to acid chloride 3.[4] The reaction gave a 13% yield of material identified as epiipomeamarone (2) and apparently no ipomeamarone. The main product was the tricyclic ketone 4. Treatment of 2 with potassium acetate and acetic anhydride gave so-called acetylisoipomeamarone, saponification of which gave material identical with that produced when natural



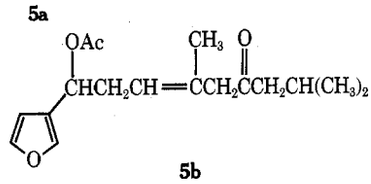
3 4

ipomeamarone was treated in the same manner. Acetylisoipomeamarone was later shown to be a mixture of cis and trans isomers **5a** and **5b**.[7a] Saponification of **5a,b** was originally thought to give only the cis-substituted tetrahydrofuran derivative **1**,[8] but a recent report indicates that the reaction actually gives a mixture of **1** and **2**.[7] Presumably both arise from **5a**; **5b** must initially isomerize to **5a** before Michael addition can occur. However, it is not clear whether **1** and **2** are formed directly from **5a** or whether one is the kinetic product and the other arises by subsequent equilibration. Regardless of the stereochemical

uncertainties of the published synthesis, the low overall yield precluded its use for synthesis of useful quantities of ipomeamarone.

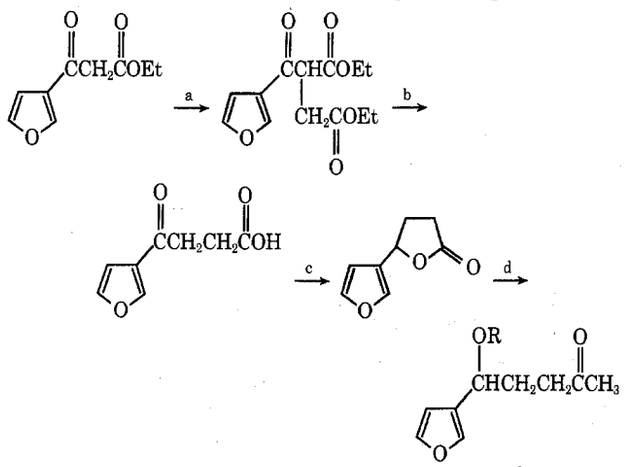


5a



5b

The present approach to the synthesis of **1** also involved **5a** and was based on the availability of 1-(3-furyl)-1-hydroxy-4-pentanone (1-ipomeanol, **6a**). 1-Ipomeanol is another of the toxic substances found in sweet potatoes and its synthesis has been outlined in a previous publication.[9]



6

a, R = H
b, R = Ac
a, NaH, DME, $BrCH_2CO_2Et$; b, H_3O^+;
c, NaOH, $NaBH_4$; d, MeLi, −78°

The condensation of the acetate derivative (**6b**) of 1-ipomeanol with the anion of dimethyl 2-oxo-4-methylpentanephosphonate produced **5a** in 80% yield.

Saponification of **5a** using Kubota's conditions gave material which the nmr spectrum indicated was an approximately equal mixture of **1** and **2**. The compounds can be distinguished in CCl_4 solution; the isopropyl methyl groups of **1** appear as two doublets whereas those of **2** appear as only one doublet.[7] Sutherland has shown that equilibration of **1** and **2** with sodium methoxide gave a 1:1 mixture of the two compounds;[7] thus a stereoselective synthesis is possible only if the initial cyclization gives only **1** or **2**.

The saponification reaction was further investigated by scanning repeatedly the nmr spectrum of a solution of **5a** in methanolic sodium hydroxide. A singlet at δ 2.12, assigned to the vinylic methyl group of **5a**, decreased in size rapidly, completely disappearing within 15 min. Simultaneously a new singlet at δ 1.35 appeared. The latter is assigned to the methyl group attached to the tetrahydrofuran rings of **1** and **2**. This process was complete within 15 min. In the isopropyl region a change from a single doublet to two approximately equal doublets was noted. This represents an equal mixture of **1** and **2**; in methanol the nmr spectra of **1** and **2** each have only one doublet for the isopropyl methyl groups, slightly displaced from one another so that mixtures of **1** and **2** exhibit four lines. After isolation of the saponification product and redissolution in CCl_4 the nmr confirmed that **1** and **2** were present in equal quantities. Natural ipomeamarone was treated with methanolic sodium hydroxide in the same fashion. After treatment for 1 hr only a trace of the epimer was present. Clearly epimerization of **1** is much slower than the formation of **1** and **2** from **5a**. It must be concluded that the cyclization process is nonstereoselective and that formation of **1** and **2** occurs at about the same rate.

In view of the failure of these attempts to achieve a stereospecific synthesis, methods for separation of the epimers were investigated. Sutherland has been able to separate **1** and **2** by analytical glpc but was unable to apply the separation on a preparative basis.[7] We readily obtained separation by high-pressure liquid chromatography on Corasil II; small-scale preparative separations were carried out with a 2.3 mm i.d. × 5 m column with ether–pentane (1:19) as eluent.

The nmr spectrum of the more rapidly eluted component was essentially the same as that reported for epiipomeamarone, while the spectrum of the other was identical with that of natural ipomeamarone. Semicarbazones were prepared from both compounds. (±)-Ipomeamarone semicarbazone melted at 114–115°. A lower melting point (109–110.5°), previously reported by Kubota,[4] was apparently for some mixture of the two epimers. The melting point of (±)-epiipomeamarone semicarbazone (102–103°) corresponded more closely to the previous report.[4]

Separations of large quantities of **1** and **2** remain impractical, but the mixture should prove useful for further toxicity studies, since it appears that all isomers have the same toxicity and physiological effect. Intraperitoneal administration of the synthetic mixture to mice indicates that it is hepatotoxic and has the same potency as the natural product obtained from sweet potatoes.

Experimental Section

Infrared spectra were obtained using a Perkin-Elmer Model 621 spectrophotometer. Nmr spectra were obtained using a Varian A-60 or JEOL JMN-MH-100 spectrophotometer with tetramethylsilane as internal standard. High-pressure liquid chromatography was carried out using a Waters Associates Model ALC202 liquid chromatograph. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. Mass spectra were obtained using an LKB gas chromatograph–mass spectrometer Type 9000. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are corrected.

1-(3-Furyl)-1-hydroxy-4-pentanone (6a). Ethyl (3-furoyl)acetate[4] (41.0 g, 0.23 mol) dissolved in 50 ml of freshly distilled dimethoxyethane (DME) was added to a stirred suspension of 5.75 g (0.25 mol) of sodium hydride in 150 ml of DME. After 10 min, 48 g (0.29 mol) of ethyl bromoacetate in 50 ml of DME was added and the mixture was stirred at room temperature overnight, then poured into 250 ml of water and extracted with ether. After drying, the ether was removed to give 100 g of crude diethyl 2-(3-furoyl)succinate, which was immediately suspended in 100 ml of 6 *N* HCl and 50 ml of acetone and heated at gentle reflux for 5 hr. After this time part of the acetone was removed and the aqueous solution was extracted repeatedly with ether. Extraction of the ether solution with saturated sodium bicarbonate, acidification of the bicarbonate solution, and extraction with ether gave 20.4 g (54%) of 3-(3-furoyl)propionic acid, mp 146–149°. Recrystallization of the material gave a light yellow solid, mp 148–150°, ir (Nujol) 3500–2300, 1710, and 1660 cm^{-1}.

Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.79. Found: C, 57.29; H, 4.69.

The 3-(3-furoyl)propionic acid (20.4 g, 0.12 mol) was dissolved in 200 ml of water containing 5 g of sodium hydroxide. To this solution was added 5.0 g (0.13 mol) of sodium borohydride in 50 ml of water containing a few drops of 10% sodium hydroxide. The mixture was stirred at room temperature for 10 min, acidified, and extracted with ether. After removal of the ether, the residue was dissolved in benzene containing a trace of *p*-toluenesulfonic acid and refluxed for 5 min. Chromatography on silica gel using

15% ether in hexane as eluent gave 15.7 g (85%) of 4-(3-furyl)-butyrolactone, ir (neat) 1770 cm^{-1}.

Anal. Calcd for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 63.02; H, 5.25.

4-(3-Furyl)butyrolactone (5.8 g, 38 mmol) was dissolved in 100 ml of anhydrous ether and cooled to −78° under a nitrogen atmosphere. Methyllithium (41 mmol) was added slowly; the mixture was stirred at −78° for 10 min and then allowed to warm to room temperature. Sufficient water was added to form two layers; the ether layer was separated, dried, and concentrated. Chromatography on basic alumina (activity V) using 10% ether in hexane as eluent gave 5.6 g (88%) of **6a**: ir (neat) 3400, 3110, 1705, and 1500 cm^{-1}; nmr (CCl_4) δ 2.22 (s, 3 H, CH_3), 2.0–2.2 (m, 2 H, 2-CH_2), 2.66 (t, J = 7 Hz, 2 H, 3-CH_2), 2.80 (broad singlet, 1 H, OH), 4.86 (t, J = 7 Hz, 1 H, 1-CH), 6.64 (m, 1 H, 4-furyl), and 7.67 (m, 2- and 5-furyl). Additional nmr signals at δ 1.6 and 5.0–5.3 indicate that the compound exists partly as a hemiketal.[10]

Anal. Calcd for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 64.16; H, 7.19.

1-Acetoxy-1-(3-furyl)-4-pentanone (1-Ipomeanol Acetate, 6b). 1-Ipomeanol (**6a**, 1.0 g, 6 mmol) was treated with 4 ml of pyridine and 1.6 ml of acetic anhydride at room temperature for 2 days. Excess reagents were removed under reduced pressure to give 1.3 g of **6b**, ir (CCl_4) 3135, 1730, and 1715 cm^{-1}.

Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.78; H, 6.82.

1-Acetoxy-1-(3-furyl)-4,8-dimethylnon-4-en-6-one (5a). Dimethyl 2-oxo-4-methylpentanephosphonate[11] was prepared from dimethyl methanephosphonate and ethyl isovalerate in a manner similar to that described by Büchi for the preparation of diethyl 2-oxopropanephosphonate:[12] nmr (CCl_4) δ 0.93 [d, J = 7 Hz, 6 H, $CH(CH_3)_2$], 2.25 [seven-line multiplet with 7 Hz separation between lines, 1 H, $CH(CH_3)_2$], 2.50 (d, J = 7 Hz, 2 H, 3-CH_2), 3.04 (d, J_{P-H} = 24 Hz, 2 H, 1-CH_2), and 3.78 (d, J_{P-H} = 11 Hz, 6 H, OCH_3). The phosphonate (2.2 g, 11 mmol) was added to 0.28 g (11 mmol) of sodium hydride suspended in 10 ml of DME. After 10 min, 1.3 g (6 mmol) of 1-ipomeanol acetate was added. The mixture was stirred at 55° under nitrogen for 36 hr. The cooled reaction mixture was chromatographed on silica gel without further work-up. Elution with 5% ether in hexane gave 1.4 g of **5a**:[7a] ir (CCl_4) 3130, 1740, 1690, and 1620 cm^{-1}; nmr (CCl_4) δ 0.92 [d, J = 7 Hz, 6 H, $CH(CH_3)_2$], 1.98 (s, 3 H, acetyl CH_3), 2.10 (broad singlet, 3 H, vinyl CH_3), 2.0–2.7 (m, 7 H), 5.70 (three-line multiplet, 1 H, 1-CH), 6.0 (two-line multiplet, 1 H, vinyl CH), 6.36 (m, 1 H, 4-furyl), and 7.40 (m, 2 H, 2- and 5-furyl); m/e 292.

Saponification of 5a. A solution of **5a** (100 mg) in 2 ml of methanol and 2 ml of 1 N sodium hydroxide was heated on a steam bath for 1 hr. Isolation by ether extraction gave material for which the nmr spectrum corresponded to that of natural ipomeamarone but had additional signals primarily in the δ 1.0 region. The two major components which were present in about equal amounts were separated by high-pressure liquid chromatography on a 5 m × 2.3 mm i.d. Corasil II column using 5% ether in pentane as eluent. The first was identified as (±)-epiipomeamarone (**2**): ir (CCl_4) 3135, 1710, 1500, 1465, 1370, 1165, 1030, 915, and 875 cm^{-1}; nmr (CCl_4) δ 0.90 [d, J = 6 Hz, 6 H, $CH(CH_3)_2$], 1.24 (s, 3 H, 4-CH_3), 1.8–2.4 (m, 7 H), 2.56 (s, 2 H, 5-CH_2), 4.82 (m, 1 H, 1-CH), 6.28 (m, 1 H, 4-furyl), and 7.32 (m, 2 H, 2- and 5-furyl). The semicarbazone of **2** melted at 102–103° after two recrystallizations from carbon tetrachloride–hexane. The second peak was identified as (±)-ipomeamarone (**1**): ir (CCl_4) 3135, 1710, 1505, 1465, 1370, 1165, 1030, 920, and 875 cm^{-1}; nmr (CCl_4) δ 0.83 (d, J = 7 Hz) and 0.85 (d, J = 7 Hz), total of 6 H [$CH(CH_3)_2$], 1.23 (s, 3 H, 4-CH_3), 1.8–2.4 (m, 7 H), 2.55 (s, 2 H, 5-CH_2), 4.86 (m, 1 H, 1-CH), 6.30 (m, 1 H, 4-furyl), and 7.32 (m, 2 H, 2- and 5-furyl). The semicarbazone of **1** melted at 114–115° after two recrystallizations from carbon tetrachloride–hexane.

A solution of 50 mg of **5a** in 0.25 ml of methanol containing 1 drop of 10% sodium hydroxide was placed in each of two nmr tubes and the saponification was followed by nmr. The vinyl methyl singlet at δ 2.12 disappeared in approximately 15 min; during the same period a new singlet was appearing at δ 1.35. A second doublet appeared in the isopropyl region. The contents of one tube were poured into a suspension of anhydrous magnesium sulfate in ether as soon as the vinyl methyl signal at δ 2.12 had disappeared. The other tube was allowed to stand at room temperature for 2 days, after which time an additional drop of base was added and the mixture was allowed to stand for an additional day. At the end of this time it was worked up in the same way as the first. In both cases the saponified material after isolation from the ethereal solution gave an nmr spectrum (CCl_4) identical with that of the 1:1 mixture of **1** and **2** described above.

Acknowledgment. This work was supported by Training Grant 5 TO1 ES00112-07 and Center in Toxicology Grant ES00267 to Vanderbilt University from U. S. Public Health Service.

Registry No.—**1**, 51703-93-6; **2**, 51703-94-7; **5a**, 51703-95-8; **6a**, 34435-70-6; **6b**, 51593-60-3; ethyl (3-furoyl)acetate, 36878-91-8; 3-(3-furoyl)propionic acid, 51593-61-4; 4-(3-furyl)butyrolactone, 51593-62-5; dimethyl 2-oxo-4-methylpentanephosphonate, 41162-17-8.

References and Notes

(1) To whom correspondence should be addressed.
(2) (2*S*,5*R*)-(+)-1-[5-(3-Furyl)tetrahydro-2-methyl-2-furyl]-4-methyl-2-pentanone.
(3) (a) M. Hiura, *Giru Nosen Gakujitsu Hokoku*, **50**, 1 (1943); (b) H. Watanabe and H. Iwata, *J. Agr. Chem. Soc. Jap.*, **26**, 180 (1952).
(4) (a) T. Kubota and T. Matsuura, *J. Chem. Soc.*, 3667 (1958); (b) T. Kubota, *Tetrahedron*, **4**, 68 (1958).
(5) The enantiomer of ipomeamarone was originally isolated from *M. laetum* Forst., the Ngalo tree, and given the trivial name ngaione.[6] The epimer **2** has been given the trivial name epingaione.[7]
(6) F. H. McDowall, *J. Chem. Soc.*, **127**, 2200 (1925).
(7) (a) B. F. Hegarty, J. R. Kelly, R. J. Park, and M. D. Sutherland, *Aust. J. Chem.*, **23**, 107 (1970); (b) W. D. Hamilton, R. J. Park, G. J. Perry, and M. D. Sutherland, *ibid.*, **26**, 375 (1973).
(8) (a) A. J. Birch, R. A. Massy-Westropp, S. E. Wright, T. Kubota, T. Matsuura, and M. D. Sutherland, *Chem. Ind. (London)*, 902 (1954); (b) C. W. Brandt and D. J. Ross, *J. Chem. Soc.*, 2778 (1959).
(9) M. R. Boyd, L. T. Burka, T. M. Harris, and B. J. Wilson, *Biochim. Biophys. Acta*, **337**, 184 (1974).
(10) The fact that **6a** exists partly as a hemiketal is not surprising; see, for example, J. Huet and J. Dreux, *C. R. Acad. Sci.*, **258**, 4570 (1964).
(11) P. A. Grieco and C. S. Pogonowski, *J. Amer. Chem. Soc.*, **95**, 3071 (1973).
(12) G. Buchi and J. E. Powell, Jr., *J. Amer. Chem. Soc.*, **92**, 3126 (1970).