3-[α-(tert-Butylamino)methyl]-5-hydroxy-m-xylene-α,α′-diol, a Selective Bronchodilator†

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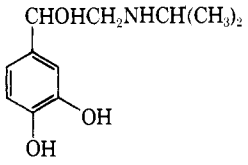
A new selective β-adrenergic stimulant 3-[α-(tert-butylamino)methyl]-5-hydroxy-m-xylene-α,α′-diol was prepared from dimethyl 5-hydroxyisophthalate in several steps. This agent possesses the structural features of salbutamol and terbutaline responsible for their selective β_2-stimulant action. It was found that this analog was selective as a β stimulant in the tracheas of dogs and guinea pigs but was less potent than either salbutamol or terbutaline.

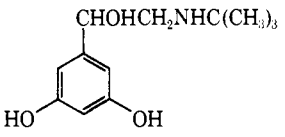
The utility of selective bronchodilators in asthma therapy is well established.[1-3] In patients with impaired lung function and those with ischemic heart disease inability to supply sufficient oxygen due to increased heart work could lead to serious cardiac insufficiency or angina. Isoproterenol is a potent nonselective β stimulant which is effective in stimulating the heart and dilating the bronchioles. Salbutamol[4] and terbutaline[5] act more selectively on the broncheolar smooth muscle in comparison with cardiac muscle. However, heart palpitations associated with salbutamol therapy continue to be a problem in a portion of patients treated.[6]

It seemed possible that combining the structural features of two selective β stimulants, salbutamol and terbutaline, into one structure such as α-[(tert-butylamino)-methyl]-5-hydroxy-m-xylene-α,α′-diol (1) might lead to further enhancement in selective β-stimulant action (Chart I).

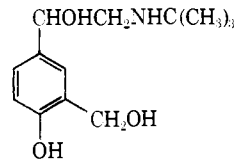
Chart I



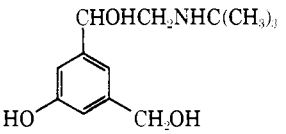
isoproterenol



terbutaline



salbutamol



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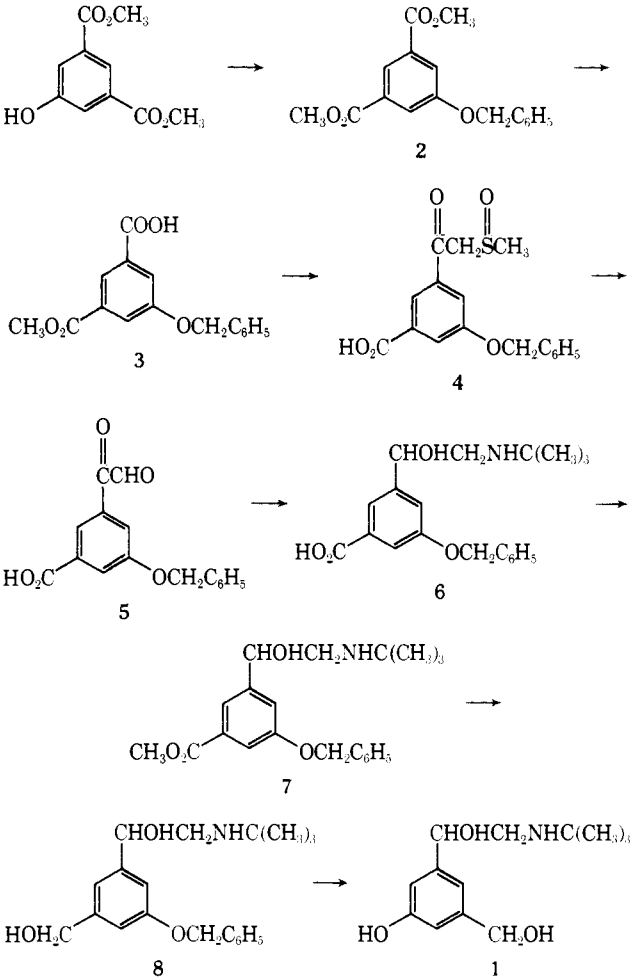
Chemistry. The synthesis of α-[(tert-butylamino)methyl]-5-hydroxy-m-xylene-α,α′-diol (1) involved the hydrolysis of dimethyl 5-benzyloxyisophthalate (2) to the acid ester 3 with 1 equiv of NaOH in methanol. The intermediate 5-benzyloxy-3-carboxy-α-(methylsulfinyl)acetophenone (4) was obtained by treatment of 3 with the dimethylsulfinyl anion in a refluxing DMSO–benzene mixture.[7] Aqueous acetic acid treatment of 4 caused a Pummerer rearrangement to 5-benzyloxy-3-carboxyphenylglyoxal (5). Reductive amination of crude 5 with tert-butylamine and KBH_4 gave 3-benzyloxy-5-[2-(tert-butylamino)-1-hydroxyethyl]benzoic acid (6). Esterification of 6 with MeOH and ethanesulfonic acid allowed a facile reduction of the methyl ester 7 with $LiAlH_4$ and gave 5-benzyloxy-α-[(tert-butylamino)methyl]-m-xylene-α,α′-diol (8) as product. Catalytic debenzylation of 8 gave the desired product 1 (Scheme I).

Scheme I



The synthesis of 1 was also accomplished by formation of methyl 5-hydroxy-3-[α-(methylsulfinyl)acetyl]benzoate (9) from dimethyl 5-hydroxyisophthalate[8] using 1 equiv of the dimethylsulfinyl anion in a benzene–DMSO mixture. A Pummerer rearrangement of 9 with dilute HCl gave the corresponding substituted phenylglyoxal 10. A reductive amination of crude 10 with tert-butylamine and KBH_4 gave the methyl 5-hydroxy-3-[2-(tert-butylamino)-1-hydroxyethyl]benzoate (11). Reduction of the ester 11 with $LiAlH_4$ gave 1 (Scheme II).

Catalytic debenzylation of 6 using 10% Pd/C and hy-

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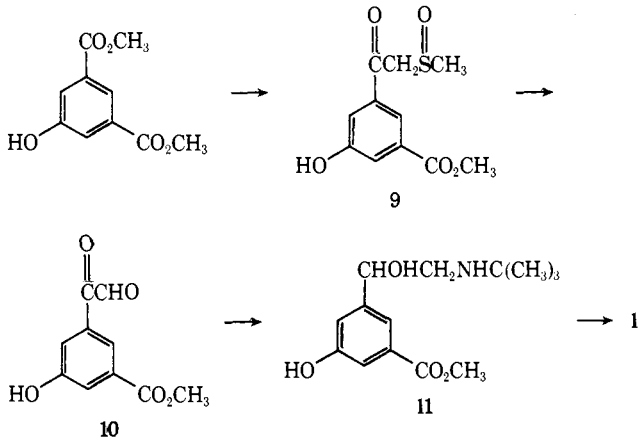
Scheme II



drogen in ethanol gave the corresponding acid analog **12**.

Pharmacology Methods. Bronchodilator activity was initially determined by antagonism of histamine-induced collapse in the guinea pig.[7] At least six male albino guinea pigs for each drug were continuously exposed for 10 min to a 0.1% histamine solution with a total dose of 4 mg aerosolized. The time from onset of aerosol to collapse of each animal was recorded. Bronchodilator drugs were administered (ip) 15 min before histamine treatment. Heart rates were determined from electrocardiograph tracings. Control values were obtained 5 and 10 min before administration of the β agonists.

Antagonism of histamine-induced changes in pulmonary resistance was studied in a minimum of four spontaneously breathing anesthetized dogs.[9,10] Air flow, transpulmonary pressure, and tidal volume signals were fed into an on-line analog computer which performed the necessary calculations for each breath. Histamine (1.2 mg) was delivered by a metered nebulizer 30 min before and 15 min after the administration (ip) of the bronchodilator drug. Protection *vs.* histamine-induced bronchoconstriction was calculated by the formula $[(a - b)/a]100$ where a equals per cent change in resistance due to histamine prior to bronchodilator administration and b equals per cent change in resistance after the administration of the drug. The maximum heart rate change produced by the β agonist before histamine administration was noted.

In vitro studies were performed using isolated guinea pig trachea and atria. Atria or spiral strips of tissue were suspended in Krebs–Hanseleit solution at 37° (95% O_2, 5% CO_2) and were connected to a Statham G10B transducer, and the contractile activity was recorded on a Grass polygraph. The strips were placed under tension and equilibrated for 2 hr prior to drug exposure. Cumulative concentration effects were obtained without change of bath medium. Regression analysis of per cent of maximum response *vs.* the log of the drug concentration provided a slope and EC_{50} (the molar concentration producing 50% of the maximum relaxation of the trachea).

The log concentration effect curves for 1 and salbutamol were less steep and reached a lower maximal efficacy than that for isoproterenol for the isolated guinea pig atria. The slope of the log drug concentration *vs.* heart rate change was 1.3 for isoproterenol, 0.62 for salbutamol and terbutaline, and 0.18 for 1. Therefore, no meaningful EC_{50} expression could be obtained. The effects of 1, terbutaline, and salbutamol upon isolated atria were evaluated by the drug concentration necessary to achieve an increased heart rate of 35 beats per minute. The maximal efficacy was assessed by comparing the maximum tracheal relaxation and atrial tachycardia following administration of each β agonist. A standard value of 1.0 was arbitrarily assigned to isoproterenol for each tissue.

In certain experiments, the β-adrenergic blocker, bunolol, 2 mg/kg ip, was administered 15 min before the β agonist.

Discussion

Replacement of the 3,4-dihydroxyphenyl moiety of isoproterenol by either 3,5-dihydroxy or 3-hydroxymethyl-4-hydroxyphenyl substitution has resulted in bronchoselective (β_2) β-adrenergic stimulant activity. The effects on β-adrenergic stimulant activity due to the incorporation of both structural modifications into one agonist structure, 3-[α-(*tert*-butylamino)methyl]-5-hydroxy-*m*-xylene-α,α'-diol (**1**), were assessed.

It was found that **1** possessed a preference for the β_2 (bronchiolar) adrenergic receptor similar to that observed for salbutamol when tested against histamine-induced bronchospasms in both guinea pigs and dogs (Table I). A study of the bronchodilator activity of **1** with the reference agents showed that **1** was approximately $\frac{1}{100}$ as potent as salbutamol and $\frac{1}{10}$ that of terbutaline using *in vivo* and *in vitro* comparisons. While the potency of **1** was lower, its maximum efficacy as a β_2 agonist was approximately equivalent to isoproterenol and salbutamol. However, the maximum efficacy of **1** in the heart was lower than salbutamol and terbutaline.

Pretreatment of guinea pigs with the β-adrenergic blocker, bunolol,[11] prevented the bronchodilator action of **1**, indicating a probable direct interaction between **1** and the β-adrenergic receptor.

Like salbutamol and terbutaline, **1** is active orally as well as by aerosol administration and possesses a long duration of action when compared with isoproterenol in experimental animals.

Experimental Section

Melting points were taken in open capillary tubes on a Mel-Temp apparatus and are uncorrected. Each analytical sample was homogeneous by tlc and had ir, uv, and nmr spectra compatible with its structure. Combustion analysis for C, H, N, Cl, and S gave results within 0.4% of theory.

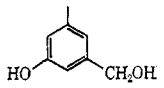
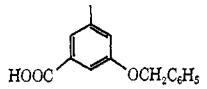
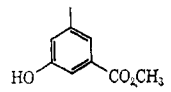
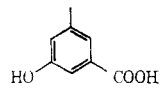
Dimethyl 5-Benzyloxyisophthalate (2). A mixture of 156 g (0.743 mol) of dimethyl 5-hydroxyisophthalate,[8] 171 g (1 mol) of benzyl bromide, and 138 g (1 mol) of K_2CO_3 in 1 l. of acetone was refluxed for 18 hr. The reaction mixture was evaporated to a residue which was extracted with hot cyclohexane to give crystalline **2** in a quantitative yield upon cooling. The analytical sample was obtained by recrystallization from cyclohexane: mp 94–95°. *Anal.* ($C_{17}H_{16}O_5$) C, H.

Methyl 5-Benzyloxyhydrogenisophthalate (3). A mixture of 91.0 g (0.304 mol) of **2** and 13.4 g (0.334 mol) of NaOH in 500 ml of MeOH was refluxed for 2.5 hr. The mixture was evaporated to a residual solid which was dissolved in EtOAc (1 l.) and washed with H_2O (2 × 500 ml). The aqueous phase was acidified with HCl and reextracted with EtOAc (3 × 500 ml). After drying the EtOAc extract of acidic phase with $MgSO_4$, evaporation of the EtOAc gave a crude white solid which was recrystallized from MeOH–H_2O to give 70.1 g (80.5%), mp 147–150°. The analytical sample was obtained by recrystallization from MeOH–H_2O: mp 154–155°. *Anal.* ($C_{16}H_{14}O_5$) C, H.

3-Benzyloxy-5-carboxy-α-(methylsulfinyl)acetophenone (4). To a mixture of DMSO (210 ml) and benzene (420 ml) heated with NaH, 57% in oil (13.0 g, 0.31 mol) for 1 hr at 75°, a DMSO solution (270 ml) containing 27.0 g (94.7 mmol) of **3** was added. The mixture was stirred at room temperature for 2 hr and then added to 3 l. of Et_2O. The Na salt was collected and dissolved in H_2O (200 ml) which was then acidified to pH 5 with HOAc. Cooling gave **4** as a yellow precipitate: yield 29.9 g (95.2%); mp 128–134°. Recrystallizations from 2-PrOH–hexane gave the analytical material: mp 137–140° dec. *Anal.* ($C_{17}H_{16}O_5S$) C, H, S.

3-Benzyloxy-5-carboxyphenylglyoxal (5). A mixture of 40.0 g (120 mmol) of **4**, 1.6 l. of 50% aqueous HOAc, and 0.8 l. of MeOH was refluxed for 68 hr. The mixture was extracted with $CHCl_3$ (1 × 3 l.). The $CHCl_3$ phase was dried with $MgSO_4$ and evaporated

Table I. Biological Results

R	Compd no.	Histamine-induced collapse in guinea pig: Dose, mg/kg	Collapse time, min	ΔHR	Histamine bronchospasm (anesthetized dog): Dose, μg/ml	% protection	ΔHR	*In vitro* (guinea pig) Trachea: EC_{50}[a]	Trachea: α[b]	Atria: EC_{35}[c]	Atria: α[b]
Saline control			2.1	20		8.7	−0.2				
Salbutamol		0.1	8.5	36	3.12	85.0	17.4	8.7×10^{-9}	0.85	3×10^{-6}	0.48
Terbutaline		1.0	5.2		12.5	71.1	36.0			3×10^{-6}	0.72
	**1**	7.5	8.3	41	300	91.3	5.0	2.8×10^{-6}	0.94	2×10^{-4}	0.31
	**6**	25	2.1								
	**11**	25	2.2								
	**12**	25	5.2								

[a]The molar concentration producing 50% of the maximum relaxation of trachea. [b]Maximum efficacy (intrinsic activity) expressed as the maximum relaxation of trachea and maximum tachycardia of atria compared to isoproterenol standard of 1.0. [c]The molar concentration of drug producing an increase in heart rate of 35 beats per minute.

to give 5 in a quantitative yield of a light yellow solid, mp 165–168°, which was purified by recrystallization from benzene–hexane: mp 167–169° dec. The crude 5 was of sufficient purity for use without further purification.

3-Benzyloxy-5-[*tert*-butylamino)-1-hydroxyethyl]benzoic Acid (6). A solution of 5 (120 mmol) and 200 ml of *tert*-butylamine in MeOH (1 l.) was stirred to 0° for 1 hr. KBH_4 (12.2 g, 228 mmol) was added at 0° in aliquots over a period of 3 hr and the resulting mixture was stirred at room temperature overnight. The mixture was evaporated *in vacuo* and the residue obtained was acidified with 3 *N* HCl (400 ml). The aqueous mixture was extracted with $CHCl_3$ (3 × 500 ml). The $CHCl_3$ extracts were combined and ether was added after cooling. A white precipitate was collected: yield 23.6 g (51.8%); mp 222–225° dec of 6 as a HCl salt. The analytical sample of 6 was obtained from 2-PrOH–Et_2O: mp 228–230° dec. The free base was obtained by stirring the HCl salt in NH_4OH. Filtration gave 15.2 g (36.9%), mp 270–272° dec. *Anal.* ($C_{20}H_{25}NO_4 \cdot HCl$) C, H, N, Cl.

3-[α-(*tert*-Butylamino)methyl]-5-hydroxy-*m*-xylene-α,α′-diol Hemifumarate (1). A mixture of 15.2 g (44.3 mmol) of 6 and 5.5 g (50 mmol) of ethanesulfonic acid was heated at reflux for 20 hr in 300 ml of MeOH. The reaction mixture was evaporated *in vacuo* to give a crude oily residue which was partitioned between $CHCl_3$ (500 ml) and 5% $NaHCO_3$ (200 ml). The $CHCl_3$ phase was dried with $MgSO_4$ and evaporated to give a methyl 3-benzyloxy-5-[2-(*tert*-butylamino)-1-hydroxyethyl]benzoate (7) as a solid residue in a quantitative yield. 7 formed a crystalline HCl salt from 2-PrOH–Et_2O, mp 192–195°, which was not further purified.

To a suspension of $LiAlH_4$ (3.42 g, 90 mmol) in 300 ml of dry THF was added a THF solution (500 ml) of 7 (14.8 g, 41.3 mmol). The reaction mixture was refluxed for 4 hr. The $LiAlH_4$ and complex were hydrolyzed by the addition of 40 ml of H_2O, slowly. The granular precipitate which formed was removed by filtration and the THF filtrate was evaporated to give 13.1 g (95.3%) of crude 5-benzyloxy-α-[*tert*-butylamino)methyl]-*m*-xylene-α,α′-diol (8) as an oil. A crystalline fumarate salt of 8 was obtained from MeOH–Et_2O: yield 10.7 g (66.8%); mp 195–200° dec.

An ethanolic solution (200 ml) containing 8 (8.25 g, 25.1 mmol) was hydrogenated over 4.0 g of 10% Pd/C catalyst until hydrogen uptake had ceased (1 hr). The catalyst was removed by filtration through a Celite pad and the EtOH filtrate was evaporated to give 1 as an oily residue: yield 5.75 g (95.9%). A crystalline hemifumarate salt was obtained from MeOH–Et_2O: yield 5.06 g (67.9%); mp 249–251° dec. The analytical sample of 1 was obtained by recrystallization from MeOH–Et_2O: yield 3.33 g (44.7%); mp 263–265° dec. *Anal.* ($C_{13}H_{21}NO_3 \cdot 0.5C_4H_4O_4$) C, H, N.

Methyl 3-[α-(Methylsulfinyl)acetyl]-5-hydroxybenzoate (9). To a cold suspension of NaH (105 mmol) in 71 ml of DMSO and 71 ml of benzene was added slowly 10.0 g (47.6 mmol) of dimethyl 5-hydroxyisophthalate[6] dissolved in 25 ml of DMSO and 25 ml of benzene. The resulting mixture was then heated at reflux for 1.5 hr, cooled, and acidified with 100 ml of 40% HOAc. The aqueous phase was separated and extracted with $CHCl_3$ (3 × 250 ml). The combined organics were dried ($MgSO_4$) and distilled off under vacuum giving a gummy residue. The crude gum was washed with hexane (2 × 20 ml) and triturated with EtOAc (10 ml) with heat. Cooling gave 4.20 g (35%) of a yellow solid, mp 144–150°. Recrystallizations from EtOAc–hexane gave the analytical sample, mp 153–155°. *Anal.* ($C_{11}H_{12}O_5S$) C, H, S.

Methyl 3-[2-(*tert*-Butylamino)-1-hydroxyethyl]-5-hydroxybenzoate (11). A reaction mixture containing 100 g (391 mmol) of 9, 1.2 l. of MeOH, and 1.2 l. of 1 *N* HCl was heated at reflux for 2 hr. The mixture was extracted with $CHCl_3$ (2 × 1 l.) and the $CHCl_3$ extract was dried with $MgSO_4$. Evaporation of the volatile components gave crude 10 in a quantitative yield.

A methanolic solution (1 l.) containing crude 10 was cooled to 0° and 500 ml of *tert*-butylamine was added. After stirring the mixture at 0° for 1 hr, KBH_4 (40.4 g, 0.75 mol) was added in four aliquots over a 3-hr period. The mixture was allowed to reach temperature and stir overnight. The reaction mixture was evaporated *in vacuo* and the residue obtained was suspended in 6 *N* HCl (750 ml). The suspension was basified with NH_4OH and extracted with $CHCl_3$ (3 × 750 ml). The $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated to give crude 11 as an oil: yield 70.6 g (67.6%). The product was purified as a hemifumarate salt from MeOH–Et_2O: mp 261–262.5° in a 30% overall yield. *Anal.* ($C_{14}H_{21}NO_4 \cdot 0.5C_4H_4O_4$) C, H, N.

3-[2-(*tert*-Butylamino)methyl]-5-hydroxy-*m*-xylene-α,α′-diol Hemifumarate (1). A THF solution (1 l.) of 11 as the free base (31.2 g, 117 mmol) was added to an ice-cold suspension of $LiAlH_4$ (8.75 g, 230 mmol) in 1 l. of THF. The resultant mixture was refluxed for 16 hr. H_2O (75 ml) was gradually added to the reaction mixture and the white solid which formed was removed by filtration. The inorganic solid was dissolved 150 ml of concentrated HCl and basified with NH_4OH. The resultant aqueous solution was extracted with EtOAc (3 × 1 l.) and the combined EtOAc extracts were dried with $MgSO_4$. The THF filtrate and EtOAc

extract were combined and evaporated to give 1 as an oil: yield 21.0 g (75.0%). A crystalline fumarate salt of 1 was obtained from MeOH-Et_2O; yield 19.5 g (55.0 %); mp 253–254° dec. The salt was recrystallized twice from MeOH-Et_2O and the analytical sample of 1 was obtained: yield 14.5 g (41.7%); mp 260–261° dec. *Anal.* ($C_{13}H_{21}NO_3 \cdot 0.5C_4H_4O_4$) C, H, N.

3-[2-(*tert*-Butylamino)-1-hydroxyethyl]-5-hydroxybenzoic Acid (12). An ethanolic solution (200 ml) containing 6.00 g (16.2 mmol) of 6 was hydrogenated over 2.5 g of 10% Pd/C catalyst at room temperature until H_2 uptake had ceased. The mixture was filtered through a Celite pad and the ethanolic filtrate was evaporated *in vacuo* to the crude 12 as the HCl salt. The analytical sample was obtained by recrystallization from EtOH-Et_2O: yield 2.15 g (44.4%); mp 188–191° dec. *Anal.* ($C_{13}H_{19}NO_4 \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl.

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Notes

Isosteres of Natural Phosphates. 3. Synthesis of the Dilithium Salt of 4,4-Diethoxy-3-hydroxybutyl-1-phosphonic Acid, an Isostere of Glyceraldehyde 3-Phosphate

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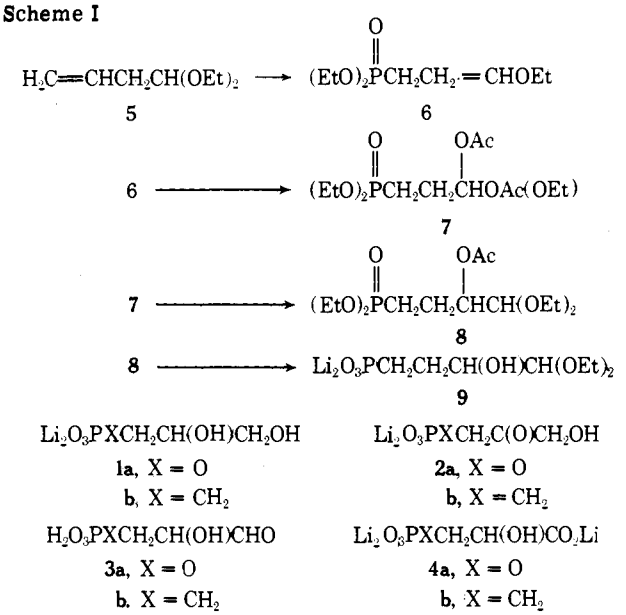
This laboratory has been engaged in the examination of the role of phosphonic acid analogs of natural organic phosphates as possible chemotherapeutic reagents which function by affecting specific biochemical pathways. We have previously reported the synthesis of 3,4-dihydroxybutyl-1-phosphonate (**1b**),[1] an analog of glycerol 3-phosphate (**1a**), and 4-hydroxy-3-oxobutyl-1-phosphonate (**2b**),[2] an analog of dihydroxyacetone phosphate (**2a**). The present report concerns the synthesis of a closely related compound, 3-hydroxy-4-oxobutyl-1-phosphonate (**3b**), an analog of glyceraldehyde 3-phosphate (**3a**). It was also expected that a precursor of **3b** would be converted readily to 3-carboxy-3-hydroxypropyl-1-phosphonate (**4b**), an isostere of 3-phosphoglyceric acid (**4a**), thereby providing a sample of this material for preliminary testing before determining whether a different, more efficient route to this isostere need be found. An alternative synthesis of **4b** has recently been reported.[3]

Results

The overall synthetic route is illustrated in Scheme I. 1,1-Diethoxy-3-butene (**5**) was chosen as a starting material because it was readily available from ethyl vinyl ether and it appeared that the four carbon unit could properly be functionalized. The initial route for the incorporation of the phosphorous group involved hydroboration and formation of the tosyl ester of the resulting alcohol, followed by an Arbuzov reaction. However, low yields and intermediates which were difficult to purify eliminated this route.

Scheme I



A free-radical addition of diethyl phosphite across the terminal double bond of the butene appeared promising. This addition was initiated by benzoyl peroxide at 85°, but it proved necessary to use a large amount of the peroxide, probably due to the presence of trace amounts of mercury in the butene. The product from this reaction was not the expected diethyl 4,4-diethoxybutyl-1-phosphonate but rather the corresponding enol ether **6**. This compound probably arises from an acid (benzoic acid) catalyzed pyrolysis of the acetal during the distillation. The enol ether was converted to diacetate **7** with the addition of bromine across the double bond followed by acetate displacement of bromide.

The diacetate **7** was subjected to vigorous acidic hydrolysis and the nmr spectrum of the resulting material indi-