When their conformational energy maps are compared (Figures 3 and 6), it is apparent that the A/B trans junction provides a higher degree of rotational freedom. Since there would be two major groups of conformers in relation to the two energy minima in IX, instead of one in VII, the probability of binding and the observed inotropic potency of IX are lower. Thus, IX is about 40% as potent as VII.

Furthermore, it would be reasonable to propose that it is this conformational factor that rendered the A/B trans cardenolide glycosides less potent than the A/B cis glycosides in general rather than the steric requirement for the cis A/B junction commonly advocated by most other research groups.

Conclusion

By use of this approach, the order of potency of each pair of cardiac glycosides can be accounted for by the fact that, kinetically, the effective concentration of those with more restricted rotations is higher although their optimal fitness to the receptor may be similar.

The important distinction between the preferred conformation of a biologically active compound in solution before binding to the receptor occurs and the actual conformation that it adopts in the drug–receptor complex should be noted. For the highly active compounds, the preferred conformation should be very similar to the binding conformation, whereas the binding conformation of the less active ones would be energetically less favorable or be within a minor population of conformers.

It should also be pointed out that the method used for PE calculation might not be able to yield very accurate energy values; however, the relative potential energy values (ΔE) obtained for a series of closely related compounds would still be able to give a reasonable approximation of the relative conformational potential energy in order to produce a qualitative measure of the relative population distribution.

With the advance of computer modelling, the conformational aspect of SAR can be handled in a more precise manner, enabling the prediction of the probable binding conformation. When used in conjunction with potential energy calculations, a rational approach to the study of the conformational factors in SAR is achieved.

Experimental Section

Inotropic activity was measured with use of isolated guinea pig left atria.[13]

Molecular modelling and superposition were carried out by using an interactive graphics program, CRYSX, running on an Evans and Sutherland picture system supported by a PDP 11/34 computer at the UCC, Sydney. The molecules examined were constructed from published crystallographic data of aglycons and sugars. The crystal structure of gomphoside was determined by X-ray crystallography.[20]

Conformational energy calculations were carried out by using a classical potential energy program, COMOL,[21,22] at 10° intervals for the two variable torsional angles, τ_1, C_2–C_3–O_3–$C_{1'}$, and τ_2, C_3–O_3–$C_{1'}$–$C_{2'}$. Since there is still no universally accepted method to satisfactorily account for the effect of solvation, the effect of hydration of the molecules in solution has been neglected.

The population distribution calculation, carried out with use of the ΔE values from the conformation energy calculation, is based on the Boltzmann's distribution function.[19] The probability of existence of each state or conformer is proportional to the term $\exp(-\Delta E_{ij}/RT)$, where ΔE_{ij} is the potential energy of the conformer, R the gas constant, and T the absolute temperature.

The Z_{ij} values are normalized to the whole conformational space. The cumulative population distribution is then calculated by integrating Z_{ij} above a computed isoprobability value such that each contour represents a boundary which encloses the specified proportion of the most likely conformers (or alternatively, the chance of finding a molecule with the conformation enclosed by this boundary at any one time).

The distance of separation calculations were carried out by defining the geometry of the superimposing functional groups (3′-hydroxyl, 5′-methyl) of gomphoside in the coordinate frame of the flexible cardiac glycoside when the aglycon part of the two cardenolides were optimally superimposed. The distances between each pair of the superimposing functional groups was computed at each 10° step of rotation about τ_1 and τ_2.

Registry No. VII, 508-93-0; VIII, 18404-43-8; IX, 34340-33-5; X, 94595-31-0.

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Synthesis and Dopaminergic Activity of (*R*)- and (*S*)-4-Hydroxy-2-(di-*n*-propylamino)indan

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A synthetic precursor to a potent dopaminergic agonist, (*RS*)-4-hydroxy-2-(di-*n*-propylamino)indan (1), has been resolved by classical recrystallization procedures, and the absolute configurations of the enantiomers have been established by X-ray crystallographic analysis. The enantiomers were converted by literature procedures into (*R*)- and (*S*)-1. (*R*)-1 was approximately 100 times as potent as (*S*)-1 in an assay for dopamine agonist effect in the isolated cat atrium.

Hacksell et al.[1] reported powerful dopamine agonist effects for (*RS*)-4-hydroxy-2-(di-*n*-propylamino)indan (1). The present structure–activity study was aimed at resolution of (*RS*)-1 and investigation of the stereochemistry of this dopaminergic agent. Our preparation of 1 differed from the literature method[1] and is shown in Scheme I.

† College of Pharmacy, University of Iowa.
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§ Eli Lilly & Co.

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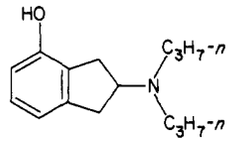

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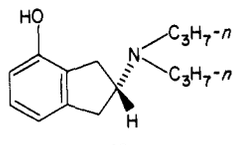
Conversion of **2** into **6** is a modification of a route devised by Barco et al.[2] It was found that the resolution could best be effected on the primary amine **8** (Scheme I), and the remainder of the steps leading to the enantiomers of **1** were performed on (*R*)- and (*S*)-**8**. Spectral data (IR, NMR, MS) on all intermediates and final products were consistent with the proposed structures.

X-ray Crystallographic Determination of Absolute Configuration. An X-ray diffraction study of the D-(−)-tartaric acid salt of **8** was undertaken in order to determine the absolute configuration of the amine with reference to the known configuration of D-(−)-tartaric acid. This analysis showed that the asymmetric carbon atom at position 2 has the *R* configuration.

Pharmacology. Results and Discussion. With in vitro cat right atrium experiments, the *R* and *S* enantiomers of **1** were found to inhibit stimulation-induced tachycardia via the presynaptic dopamine receptors. In all experiments, drug-induced inhibitions were reversed by a dopamine antagonist, haloperidol (50 μg/L), but not by an α_2-adrenoceptor antagonist, yohimbine (100 μg/L). ID_{50} values with 95% confidence intervals of the *R* and *S* enantiomers were determined to be 0.007 (0.006–0.009) μM and 0.720 (0.609–0.865) μM, respectively. Dopamine receptor stimulating potency of the *R* enantiomer was thus approximately 100 times that of the *S* enantiomer. The ID_{50} of apomorphine in this assay was found to be 0.039 (0.007–0.073) μM.

Structure–Activity Discussion. The more potent enantiomer of **1**, **10**, has the *R* absolute configuration. This stereochemistry coincides with that of the more active enantiomers of apomorphine (**11**),[3] 5-hydroxy- and 5,6-dihydroxy-2-aminotetralin (**12** and **13**),[4] and 6-hydroxyoctahydrobenzo[*f*]quinoline (**14**).[5] Thus, the optical consistency noted[6] for chiral dopaminergic agonists having the benzene ring substitution pattern as in **10**–**14** is maintained.



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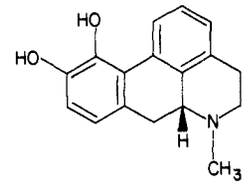

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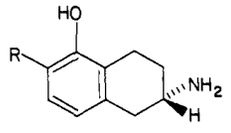
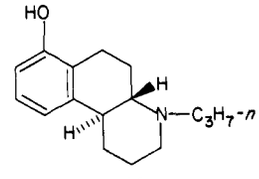


12, R=H
13, R=OH



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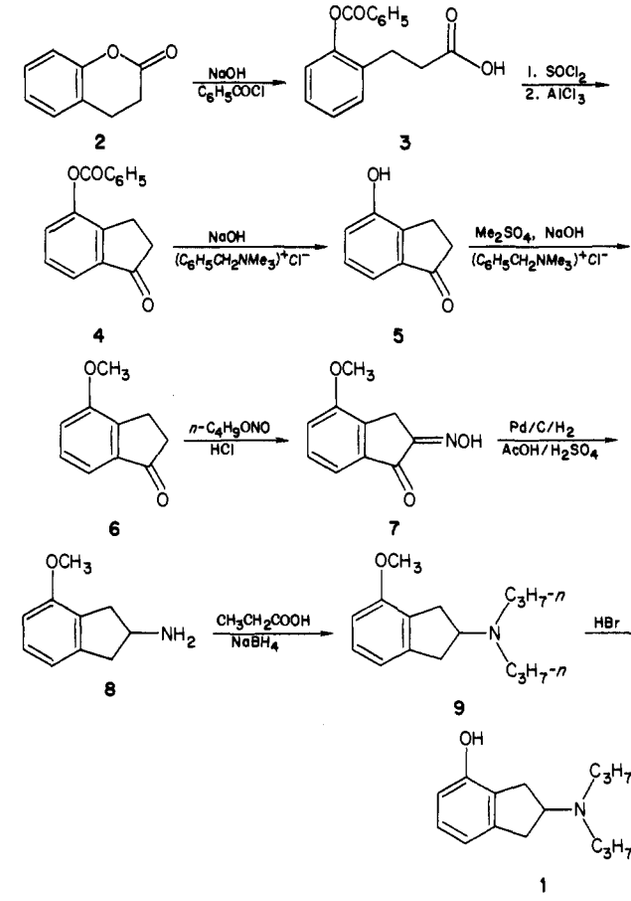
Scheme I. Preparation of 4-Hydroxy-2-(di-*n*-propylamino)indan



It is noted that in compound **8**, which was used for the X-ray crystallographic study, the cyclopentene ring is not planar (in accord with previous NMR studies[7,8]), and the torsion angle N–C2–C1–C7′ (structure **15**) is −90.0°. Thus, the amino group is in a pseudoaxial disposition and the *N*–C2 bond is nearly perpendicular to the plane of the benzene ring (structure **16**). This conformation contrasts with the torsion angle 140–150° (illustrated in **17**), which we have speculated[9] to be the biologically significant one for dopaminergic 2-aminoindans. However, it cannot be assessed whether the conformation observed in the crystalline state for the D-(−)-tartrate salt of a methyl ether derivative of a primary amine has any relevance to the solution conformation of the free phenolic *N*,*N*-di-*n*-propyl tertiary amine when it interacts with in vivo dopamine receptors.

Experimental Section

Pharmacology. Methods. Isolated Cat Right Atria. Cats of either sex (2–4 kg) were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). Following midline thoractomy, the heart was quickly excised. The right atrium was dissected free of ventricular muscle and left atrium in oxygenated Feigen solution (mM) NaCl, 154.0; KCl, 5.6; $NaHCO_3$, 23.8; glucose, 11.1; and $CaCl_2$, 5.6 dissolved in glass-distilled H_2O. The right atrium was suspended between two Pt electrodes in a 100-mL organ bath containing Feigen solution. The tissue bath was

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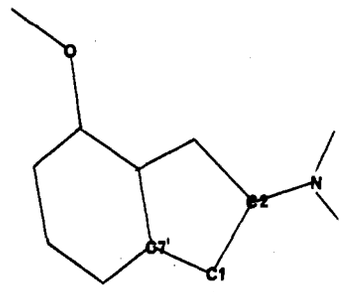

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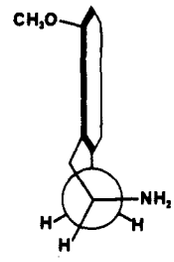

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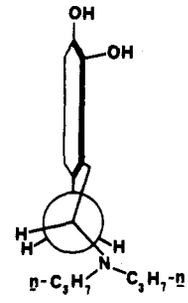

17

maintained at 37 °C and was aerated with 95% O_2–5% CO_2. The right atrium was attached by a ligature to a force displacement transducer. Atrial rate was measured with a Beckman cardiotachometer and a Beckman recorder. Resting tension was adjusted to 1 g, and the preparation was allowed to equilibrate for 30 min, with the Feigen solution being changed every 10 min. The right atrium was then stimulated transmurally in every 5 min (100 V, 5-ms duration for 10 s) with a Grass stimulator. Stimulations were performed at 2 Hz. The test compounds were added in increasing concentrations (0.3 log unit).

Chemistry. Melting points were determined in open glass capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated by the symbols of the elements, analytical results were within ±0.4% of the theoretical values.

3-[2-(Benzoyloxy)phenyl]propionic Acid (3). Dihydrocoumarin (**2**; 74 g, 0.5 mol) in 133 mL of 30% NaOH was stirred at room temperature and benzoyl chloride (70 g, 0.5 mol) was added dropwise over 30 min. The resulting solution was cooled in ice–H_2O and was acidified with concentrated HCl. The solid which separated was collected on a filter and dried under reduced pressure at 80 °C. Recrystallization from toluene gave 84.5 g (63%) of fine white needles, mp 146 °C (lit.[2] mp 140 °C).

4-(Benzoyloxy)-1-indanone (4). Thionyl chloride (75 g, 0.63 mol) was added dropwise to a stirred suspension of 67.5 g (0.25 mol) of **3** in 500 mL of CH_2Cl_2 containing a few drops of DMF. The mixture was heated under reflux for 4 h. The solvent was then removed under reduced pressure, and excess thionyl chloride was removed by azeotroping with several portions of benzene (dried over 4-Å molecular sieves). The residual yellow oil was dissolved in 100 mL of CH_2Cl_2 and this solution was added dropwise to a stirred suspension of 150 g (1.1 mol) of $AlCl_3$ in 100 mL of CH_2Cl_2. The mixture was then heated under reflux for 3 h and was then cautiously poured over 500 mL of ice–H_2O slurry. The resulting mixture was filtered, the filtrate separated into two layers, and the aqueous layer was extracted with CH_2Cl_2. The pooled organic phases were washed with 5% $NaHCO_3$ and then with H_2O, dried ($MgSO_4$), and evaporated to leave 58.1 g (92%) of a brown oil. Trituration of this with cold Et_2O gave a solid, which was recrystallized from Et_2O. The mother liquor was chromatographed on silica and eluted with Et_2O to provide additional product: total yield, 47.4 g (75%) of off-white platelets; mp 85 °C (lit.[2] mp 85 °C).

4-Hydroxy-1-indanone (5). A mixture of 20 g (0.08 mol) of **4**, 12.4 g (0.04 mol) of benzyltrimethylammonium chloride, 450 mL of CH_2Cl_2, and 450 mL of 1.0 N NaOH was stirred under reflux for 4 h. The layers were separated, and the organic layer was washed with 0.5 N HCl then with 0.5 N NaOH. The basic extracts were acidified with concentrated HCl, and the solid which separated was collected on a filter. This material was washed with copious amounts of 5% $NaHCO_3$ and was then dried under reduced pressure at 80 °C overnight to yield 8.4 g (72%) of a pale yellow powder, mp 230–234 °C (lit.[10] mp 239–240 °C). Recrystallization from MeOH gave 6.5 g (55%) of product, mp 244 °C. The aqueous mother liquor from the original precipitation of the crude product was extracted with CH_2Cl_2, and from this extract was isolated, after recrystallization from MeOH, an additional 0.5 g of product: mp 244 °C; total yield, 7.0 g (60%).

4-Methoxy-1-indanone (6). A procedure of McKillop et al.[11] was employed. A mixture of 6.29 g (42.5 mmol) of **5**, 250 mL of CH_2Cl_2, 250 mL of 1.2% NaOH, 1.5 g of benzyltrimethylammonium chloride, and 11.9 g (94.4 mmol) of dimethyl sulfate was stirred at room temperature for 1 h. An additional 5.3 g (42.3 mmol) of dimethyl sulfate was added, and stirring was continued for 1 h more. Excess dimethyl sulfate was then destroyed by addition of 90 mL of concentrated NH_4OH and stirring at room temperature for 1 h. The two layers were separated, and the aqueous layer was extracted with two 250-mL portions of CH_2Cl_2, which were pooled with the organic layer of the reaction mixture. The organic solution was washed with two 150-mL portions of H_2O. The aqueous extracts were washed with CH_2Cl_2, and this extract was added to the organic phase. The combined organic solution was dried ($MgSO_4$) and evaporated to leave a light brown oil, which was distilled from a Kugelrohr apparatus, bp 116–120 °C (0.3 mm). The colorless distillate solidified upon standing, and it was recrystallized from MeOH to provide 6.7 g (97%) of white needles, mp 103–104 °C (lit.[10] mp 102–103 °C).

4-Methoxy-2-oximino-1-indanone (7). Freshly distilled *n*-butyl nitrite (3.5 g, 34 mmol) was added to a solution of 5.0 g (31 mmol) of **6** in 250 mL of a saturated solution of anhydrous HCl in Et_2O. The resulting deep red solution was stirred at room temperature for 1 h and then it was allowed to stand at 0 °C for 2 h. The solid which separated was collected on a filter, washed with anhydrous Et_2O, and air-dried to give 5.6 g (95%) of a fine yellow powder, mp 225–227 °C. An analytical sample was recrystallized twice from EtOH, mp 236 °C. Anal. ($C_{10}H_9NO_3$) C, H, N.

(±)-2-Amino-4-methoxyindan (8). Compound **7** (14.1 g, 74 mmol) was hydrogenated in 270 mL of AcOH and 18 mL of concentrated H_2SO_4 over 7.0 g of 5% Pd/C at room temperature at an initial pressure of 55 psig. When no more H_2 was taken up, the reduction mixture was filtered and the filtrate was concentrated under reduced pressure. This residue was taken to pH 10 (pH paper) with 30% NaOH and was then extracted with five 200-mL portions of Et_2O. The pooled extracts were taken to dryness, and the residue was dissolved in CH_2Cl_2 and this solution was dried (Na_2SO_4). Evaporation of the solvent left a brown oil: bp 87–90 °C (0.05 mm) (lit.[1] bp 105–107 °C (0.1 mm)); yield, 8.6 g (72%) of a colorless oil. A portion of this material was converted to its HCl salt, mp 236–237 °C (2-PrOH–Et_2O) (lit.[1] mp 240–241 °C).

Resolution of (±)-2-Amino-4-methoxyindan (8). Compound **8** was resolved by fractional crystallization of the diastereomeric

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salts of both D-(−)- and L-(+)-tartaric acids. Crystallizations of the L-(+)-tartrate salts gave (*S*)-8 as the principal component of the less soluble diastereomeric mixture, while crystallization of the D-(−)-tartrate salts produced (*R*)-8. Freshly distilled (±)-8 (8.6 g, 53 mmol) in 500 mL of MeOH was combined with 7.9 g (53 mmol) of L-(+)-tartaric acid in 500 mL of MeOH. After 2 days, the crystals which separated were collected and recrystallized from MeOH. The mother liquor from the original crystallization was evaporated to dryness, and the free base was regenerated from the residue. This material in 400 mL of MeOH was treated with an equivalent weight of D-(−)-tartaric acid in 400 mL of MeOH. After 2 days, the crystals which separated were collected and recrystallized from MeOH. Both enantiomeric salts were repeatedly recrystallized from MeOH to constant optical rotation to provide (*S*)-8 L-(+)-tartrate [5.8 g, 70%; colorless prisms, mp 224–226 °C; $[\alpha]^{24}_D$ + 9.764° (free base, $CHCl_3$, *c* 11.88)] and (*R*)-8 D-(−)-tartrate [6.2 g, 75%; colorless prisms, mp 224–226 °C; $[\alpha]^{24}_D$ −9.647° (free base, $CHCl_3$, *c* 13.17)].

X-ray Diffraction Study of the D-(−)-Tartrate Salt of (−)-2-Amino-4-methoxyindan (8). The compound crystallizes in the noncentrosymmetric, triclinic space group, *P1*, with one molecule in a unit cell having the following dimensions: a = 9.937 Å, b = 7.479 (2) Å, c = 5.399 (1) Å, α = 77.99 (2)°, β = 104.15 (2)°, and γ = 78.35 (2)°. The density calculated for $(C_{10}H_{14}NO)^+(C_4H_5O_6)^-$, Mr = 313.3, is 1.42 g cm^{-3}. The intensities of 1106 unique reflection were measured on a computer automated four-angle diffractometer using monochromatic copper radiation. The structure was solved by direct methods (SHELXTL) and refined by the least-squares method, with anisotropic temperature factors for all non-hydrogen atoms. The hydrogen atoms were all located from a difference *E* map but were included in the final refinement at calculated positions. Figure 1 and Tables I–V (supplementary material) show an ORTEP plot of the molecule and give atom coordinates, anisotropic temperature factors, bond lengths, bond angles, and hydrogen coordinates.

(*R*)- and (*S*)-2-(*Di-n*-propylamino)-4-methoxyindan Hydrochloride (9). The free bases of (+)- and (−)-8 were alkylated by a method of Marchini et al.[12] The appropriate free base (1.3 g, 7.98 mmol) in 5 mL of dry benzene was added to a previously prepared solution of 3.0 g (79.8 mmol) of $NaBH_4$ and 19.5 g (260 mmol) of propionic acid in 50 mL of benzene (dried over 4-Å molecular sieves), and the resulting mixture was heated under reflux under N_2 for 20 h. The cooled reaction mixture was then washed with two 100-mL portions of 2 N NaOH, and the organic layer was dried (Na_2SO_4) and evaporated to leave an almost colorless oil. Treatment of this with ethereal HCl deposited a solid which was recrystallized from 2-PrOH–Et_2O. (*R*)-9·HCl: yield, 1.99 g (88%); mp 189–190 °C (lit.[1] mp for racemic mixture 189–190 °C); $[\alpha]^{23}_D$ −14.423° (HCl salt, EtOH, *c* 10.40). (*S*)-9·HCl: yield, 2.0 g (90%); mp 189–190 °C (lit.[1] mp for racemic mixture 189–190 °C); $[\alpha]^{23}_D$ +14.422° (HCl salt, EtOH, *c* 9.99).

(*R*)- and (*S*)-2-(Di-*n*-propylamino)-4-hydroxyindan Hydrobromide (1). The appropriate enantiomeric HCl salt (3.0 g, 10.6 mmol) in 30 mL of 48% HBr was heated under N_2 at 125 °C for 3 h. The reaction mixture was evaporated under reduced pressure and H_2O was removed by repeated azeotroping with benzene. The solid residue was recrystallized from 2-PrOH and then from EtOH–Et_2O. (*S*)-1·HBr: yield, 3.06 g (92%); mp 225–226 °C (lit.[1] mp for racemic mixture 204–205 °C; $[\alpha]^{23}_D$ + 13.379° (HBr salt, EtOH, *c* 10.17). (*R*)-1·HBr: yield, 3.18 g (96%); mp 224–225 °C (lit.[1] mp of racemic mixture of 204–205 °C; $[\alpha]^{23}_D$ −13.353° (HBr salt, EtOH, *c* 10.34).

Acknowledgment. This work was supported in part by Grant GM-22365, National Institute of General Medical Sciences, and in part by a grant from Eli Lilly and Co.

Registry No. (*R*)-1, 94843-89-7; (*S*)-1, 94843-90-0; (*R*)-1·HBr, 94843-91-1; (*S*)-1·HBr, 94843-92-2; **2**, 119-84-6; **3**, 59725-59-6; **3** (acid chloride), 59725-62-1; **4**, 59725-61-0; **5**, 40731-98-4; **6**, 13336-31-7; **7**, 24623-28-7; (*R*)-**8**, 94903-37-4; (*S*)-**8**, 94903-38-5; (*R*)-8·D-(−)-tartrate, 94903-39-6; (*S*)-8·L-(+)-tartrate, 94903-40-9; (*R*)-9·HCl, 94859-22-0; (*S*)-9·HCl, 94859-23-1; (±)-11, 94843-93-3; benzoyl chloride, 98-88-4; propionic acid, 79-09-4.

Supplementary Material Available: Figure 1 showing an ORTEP plot of (*R*)-8 and Tables I–V listing atom coordinates and temperature factors, anisotropic temperature factor, bond lengths, bond angles, and hydrogen coordinates and temperature factors (4 pages). Ordering information is given on any current masthead page.

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Synthesis and β-Lactamase Inhibitory Properties of 2β-[(Acyloxy)methyl]-2-methylpenam-3α-carboxylic Acid 1,1-Dioxides

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p-Nitrobenzyl 2β-[(benzoyloxy)methyl]-2α-methylpenam-3α-carboxylate was prepared by reaction of *p*-nitrobenzyl 2-[2-oxo-3α-bromo-4-(benzothiazol-2-yldithio)azetidin-1-yl]-2-isopropenylacetate with silver benzoate in the presence of iodine. The resulting diester was oxidized to the sulfone with potassium permanganate and hydrogen peroxide, and the bromine and *p*-nitrobenzyl groups were removed by hydrogenolysis to give potassium 2β-(benzoyloxy)methyl 2α-methylpenam-3α-carboxylate 1,1-dioxide. A series of related compounds, including the pivaloyl, methoxybenzoyl, *p*-fluorobenzoyl, and *p*-aminobenzoyl derivatives, were prepared in a similar way. All of these compounds were potent β-lactamase inhibitors in vitro against the TEM β-lactamase from *Klebsiella pneumoniae* A22695 and *Bacteroides fragiles* A22695 but less active against the β-lactamase from *Staphylococcus aureus* A9606. All compounds when administered orally in a 1:1 combination with amoxicillin did not show any significant protection of mice infected with *S. aureus* A9606. 2β-(Bromomethyl)-2α-methylpenam-3α-carboxylic acid was prepared and reacted with silver nitrate to give the nitrate ester. Oxidation with potassium permanganate and catalytic reduction afforded 2β-(hydroxymethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide. 2β-(Bromomethyl)-2α-methylpenam-3α-carboxylic acid 1,1-dioxide was found to be a strong β-lactamase inhibitor, while the 2β-hydroxymethyl compound showed only weak β-lactamase-inhibiting properties.

The discovery of the β-lactamase inhibitory properties of penicillanic acid sulfone (sulbactam[1]) has led us to the investigation of this activity in a number of other relatively simple semisynthetic derivatives of 6-aminopenicillanic acid.[2] Among the most active of these derivatives was 2β-(chloromethyl)-2α-methylpenam-3α-carboxylic acid

(1) Aswapokee, N.; Neu, H. C. *J. Antibiot.* **1978**, *31*, 1238.

(2) For leading references, see ref 3; Claverley, M. J.; Begtrup, M. J. *Antibiot.* **1983**, *26*, 1507. Arisawa, M.; Then, R. L. *J. Antibiot.* **1983**, *26*, 1372.

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