on a receptor in its preferred conformation, but rather that molecular complimentarity to the receptor must be sufficiently probable conformationally for a molecule to form a complex with the receptor. It is with this in mind that the preceding conformational and structural model of peptic ulcer therapy has been presented.

Appendix A

The basic computational results upon which the conformational analysis of 2-phenyl-2-(2-pyridyl)thioacetamide has been based are included in Tables III-VI. The angle definitions and initial positions of the $CS(NH_2)$, pyridyl, and phenyl groups are defined by structure **10** and the accompanying discussion. The minima at $\theta_2 = 103$ and 277° were estimated from the results in Table III by parabolic interpolation and the more refined calculations were based on these values. The tabular energies are related to the EHT calculated energies by $E = E(\text{calcd}) + 33{,}400$ kcal/mole in order to make the energy variation more obvious.

Appendix B

The computational results upon which the conformational analysis of phenylalanine amide has been based are summarized in Tables VII-IX. The angle θ_1 and its initial position at 255° are defined by **15**, and θ_2 and its initial position at 0° are defined by **16**. The variation of E *vs.* θ_1 at $\theta_2 = 110°$ (the estimated local minimum for this variable) has not been performed as thoroughly as that appearing in Tables VIII and IX. However, this rotamer would contribute minimally to the population since $E = 1.4$ kcal/mole at $\theta_1 = 255°$, while from Tables VIII and IX, the corresponding minima are −2.5 kcal/mole and −1.8 kcal/mole, respectively. The E above and in the tables is related to the calculated EHT energies by $E = E(\text{calcd}) + 26{,}900$ kcal/mole.

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Semisynthetic β-Lactam Antibiotics. 1. Acylation of 6-Aminopenicillanic Acid with Activated Derivatives of α-Sulfophenylacetic Acid[1]

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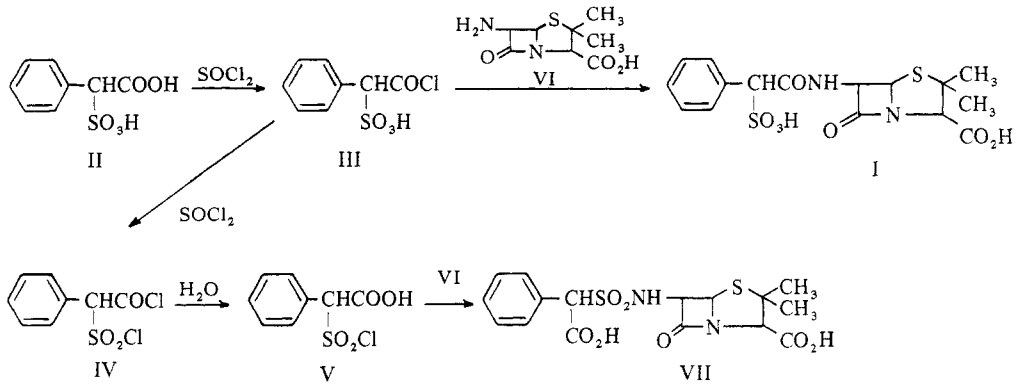
α-Sulfobenzylpenicillin is a new semisynthetic penicillin with a wide-spectrum activity against Gram-positive and Gram-negative bacteria and peculiar in possessing a potent inhibitory effect against *Pseudomonas aeruginosa*. Acylation of 6-aminopenicillanic acid (6-APA, VI) by using a new activated derivative of α-sulfophenylacetic acid, *i.e.*, α-sulfophenylacetyl chloride(III), yielded α-sulfobenzylpenicillin (I). In contrast, the reaction of 6-APA with α-chlorosulfonyl-α-phenylacetic acid (V) gave another penicillin corresponding to the isomer of I, namely, 6-(α-carboxy-α-toluenesulfonamido)penicillanic acid (VII), which showed poor results as expected from the same kind of penicillin having a SO_2NH side chain at the 6 position.

In the course of a study on semisynthetic penicillins, we became interested in the preparation of a new penicillin derived from α-sulfophenylacetic acid and in examining whether the α-sulfoacyl side chain would confer physicochemical and biological properties on the penicillin molecule different from those conferred by the α-carboxyacyl side chain. As compared to the α-carboxyl group of carbenicillin, the sulfo group of α-sulfobenzylpenicillin (I) is stronger in acidity and larger in size, consisting of highly polar hetero atoms, sulfur and oxygen. A difference, dependent on the

Table I. Nmr and Ir Data for α-Sulfophenylacetic Acid and Its Acid Chlorides

Compound	Nmr (60 Mcps) (solvent, methine phenyl), ppm			Ir (KBr), cm^{-1}						
				C=O				SO_2		
$C_6H_5C(SO_3H)HCOOH$	D_2O	4.98, s	7.3–7.8, m			1730		1265	1174	1035
$C_6H_5C(SO_3H)HCOCl$	$CDCl_3$	5.57, s	7.40, s	1800	1770			1260	1180	1041
$C_6H_5C(SO_2Cl)HCOCl$	$CDCl_3$	5.90, s	7.50, s	1790			1385		1175	
$C_6H_5C(SO_2Cl)HCOOH$	$CDCl_3$	5.58, s	7.45, br s			1730	1370		1160	
$C(SO_2Cl)H_2COOH$	$CDCl_3$	4.82				1720	1370		1165	1039

Scheme I



acidities, the polarities, and the spatial sizes of the groups, might be expected in their affinities for active sites on enzymes.[2] Actually, the biological testing was rewarding and proved that I (DL form) is a highly potent, broad-spectrum penicillin comparable to carbenicillin. This paper deals with the syntheses of α-sulfobenzylpenicillin by the reaction of 6-aminopenicillanic acid (6-APA, VI) with α-sulfophenylacetyl chloride (III) and with that of 6-(α-carboxy-α-phenylmethanesulfonamido)penicillanic acid (VII).

α-Sulfophenylacetic Acid and Its Chlorides. α-Sulfophenylacetic acid (II), the starting compound for our synthesis, has in the past received very little attention. The first synthesis was achieved by Brust[3] in 1927 by the reaction of α-bromophenylacetic acid with NH_4HSO_3. A more efficient synthesis was noted in 1953 when Truce studied the reaction of phenylacetic acid with SO_3 in CH_2Cl_2.[4] Although II has been known for over 40 years, no functional derivative of the carboxyl group has yet been reported in the literature.

The preparation of the common types of activated derivatives of the carboxyl group in II, *e.g.*, the acid chloride, the mixed anhydride, and the activated ester, is essential for the preparation of the present penicillin. α-Sulfophenylacetic acid which contains two acidic groups on the same carbon atom can be converted theoretically into two mono- and one difunctional derivative and, therefore, detailed attempts were made for the selective preparation of α-sulfophenylacetyl chloride (III) using a variety of reagents and reaction conditions. As a result, conversion into III was successfully achieved by treating dried α-sulfophenylacetic acid with excess $SOCl_2$ in the presence of the minimum quantity of Et_2O or another organic solvent in the temperature range 15–50°. The replacement reaction at the carboxyl hydroxyl progressed selectively and quantitatively within several hours. After prolonged reaction for 50 hr at 20°, the remaining sulfohydroxyl group of III was subsequently substituted by chlorine to yield α-chlorosulfonylphenylacetyl chloride (IV). Compounds III and IV were found to be easily and unequivocally differentiated on the basis of nmr spectra which are useful to monitor the reaction progress (Table I). Partial hydrolysis of the diacid chloride (IV) in the presence of 1 equiv of water yielded the sulfonyl chloride (V), which is isomeric with III. Although the nmr spectrum of the methine proton shows peaks coincidentally at 5.58, V gives peaks in the ir spectrum at 1730, 1380, and 1215 cm^{-1}, indicating the presence of COOH and SO_2, respectively, but no peak at about 1050 cm^{-1}, suggesting the absence of $-SO_3H$. These reactions are summarized in Scheme I.

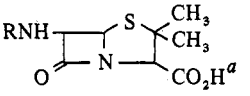
α-Sulfobenzylpenicillin. The coupling reaction of 6-APA with α-sulfophenylacetyl chloride (DL-III) was carried out in an aqueous solution containing a weak base such as $NaHCO_3$ or in an anhydrous organic solvent containing Et_3N. The acid chloride (racemic form, DL-III) was relatively stable under the conditions of the Schotten–Baumann reaction. The resulting product was best purified by chromatography on a column of Amberlite XAD-2 using water as the eluent. Lyophilization of the penicillin fraction and subsequent crystallization from ethanol gave α-sulfobenzylpenicillin (I, DL form) as colorless needles.

In the nmr spectra of DL-I some complexity arises from the existence of a pair of diastereoisomers (D- and L-I).[5] The resonances for the hydrogens of the two methyl groups occur as an apparent triplet, centered at δ 1.76 ppm, which results from the superposition of two of the four signals due to 2α- and 2β-methyl† hydrogens which are located at δ 1.76, 1.84 and at 1.70, 1.76 ppm corresponding to D- and L-I, respectively. Similarly, other protons attached to the penicillin nucleus also show pairs of signals corresponding to D- and L-I. The nmr analysis of each pure diastereoisomer will be reported in the following paper.

Another penicillin isomeric with I was synthesized by treating 6-APA with the sulfonyl chloride (V). Although penicillins prepared from sulfonic acids are in general less active than those derived from carboxylic acids,[7] no example is known in the literature of a penicillin with an α-carboxyl in the sulfonic acid side chain at the 6 position.

†The assignment was performed by the NOE method according to the technique described by Cooper.[6] Upon irradiation of the low-field methyl protons, signals for H_3 were enhanced both in I_D (34%) and I_L (27%). Alternatively, saturation of the high-field methyl increased the intensity of H_3 (22.6% for I_D, 16% for I_L). Hence, the low-field methyls in nmr spectra of I_D and I_L were assigned to the 2β-methyl protons.

Table II. Antibacterial Activities



Test organism	MIC, μg/ml			
	DL-$C_6H_5C(SO_3H)HCO$-	DL-$C_6H_5C(CO_2H)HSO_2$-	DL-$C_6H_5C(CO_2H)HCO$-	$C_6H_5CH_2SO_2$-
Pseudomonas aeruginosa IFO 3080	25	>100	25	>100
P. aeruginosa IFO 3448	25	>100	50	>100
Escherichia coli IFO 3044	25	12.5	12.5	>100
Proteus vulgaris IFO 3045	3.13	>100	1.56	>100
Pr. morganii IFO 3848	1.56	20	1.56	>100
Pr. mirabilis IFO 12255	1.56	>100	1.56	>100
Staphylococcus aureus FDA 209P	0.78	0.78	0.39	0.78
Staph. aureus Pc-R	12.5	>100	50	>100
Bacillus subtilis PCI 219	0.2	0.20	0.10	0.39
Sarcina lutea PCI 1001	0.39	0.10	0.05	0.20

[a] Used as the sodium salt.

The compound can be thought of as a sulfonic-type penicillin corresponding to carbenicillin. Chromatography of the reaction mixture and successive work-up gave 6-(α-carboxyl-α-toluenesulfonamido)penicillanic acid (VII), the structure of which was determined by ir and nmr spectra. Although VII showed relatively poor antibacterial effects and is no exception to the general rule, it is effective against a wider range of Gram-negative bacteria than is 6-(α-toluenesulfonamido)penicillanic acid (Table II).

α-Sulfobenzylpenicillin and carbenicillin possess many properties in common since they are closely related in structure and their antibacterial spectra are essentially the same, although differing in relative potency against each bacterium. However, α-sulfobenzylpenicillin (DL-I) has characteristic activity against *Pseudomonas aeruginosa* and penicillin G resistant *Staphylococcus aureus* as shown in Table II. The MIC values were found to be comparable to those of carbenicillin. Similar results were obtained by the biological research group in our laboratory.[8]

The synthesis of each diastereoisomer, D- and L-I, the comparison of the antibacterial activity, and the stability with those of carbenicillin will be reported in the following paper.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. Nmr spectra were obtained on a Varian A-60 and an A-100 spectrometer. Ir spectra were run using a Hitachi ir spectrometer (EPU-2A).

α-Sulfophenylacetyl Chloride (DL-III). DL-α-Sulfophenylacetic acid (DL-II, $C_8H_8O_5S \cdot 2H_2O$, 8.5 g, 3.38×10^{-2} mole) was added portionwise to a solution of ether (8.5 ml) and thionyl chloride (32.7 g, 0.275 mole) in a 100-ml three-necked flask containing a thermometer, a sealed stirrer, and a calcium chloride drying tube. The mixture was stirred efficiently at room temperature until gas evolution of HCl and SO_2 ceased. Then, dimethylformamide (0.2 ml) was added and the resulting solution was warmed at 40° for 4 hr with stirring. The reaction mixture was diluted to 60 ml with ether, followed by the addition of hexane (60 ml), and cooled to below −25° in a Dry Ice bath for 8 hr. Slightly yellowish crystals deposited, and, by decantation of the mother liquor, the crystalline solid was collected. After washing twice with a small amount of ether while cooling in a Dry Ice bath and then drying in a vacuum desiccator over P_2O_5, a hygroscopic crystalline powder was obtained: yield 5.7 g (72% from II). *Anal.* ($C_8H_7O_4ClS \cdot 0.5C_2H_5OC_2H_5$) C, H, S. The ir and nmr data are shown in Table I.

α-Chlorosulfonyl-α-phenylacetyl Chloride (IV). To a solution of ether (50 ml) and freshly distilled thionyl chloride (55 g, 0.463 mole) in a three-necked flask fitted with a stirrer and a calcium chloride drying tube were added α-sulfophenylacetic acid (10 g, 4.63×10^{-2} mole) and dimethylformamide (0.3 ml) with stirring. The mixture was allowed to react for 50 hr at 20°. After excess thionyl chloride was removed *in vacuo*, the residual oil was left in a refrigerator to afford colorless needles. Reprecipitation from $CHCl_3$–ether under cooling yielded 11.5 g of crystalline powder. *Anal.* ($C_8H_6Cl_2O_3S$) Cl: calcd, 28.01; found, 26.50. The ir and nmr data are shown in Table I.

α-Chlorosulfonyl-α-phenylacetic Acid (V). A solution of 26 g of α-chlorosulfonylphenylacetyl chloride (IV, 0.104 mole) in 180 ml of C_6H_6 was placed in a flask protected from moisture and cooled in an ice bath. Over a 30-min period 1.88 g (0.104 mole) of water diluted in acetone (50 ml) was added with stirring. The resulting solution was stirred for 3 hr and evaporated *in vacuo* to yield a colorless oil (20 g) which, after being stored in a refrigerator, changed to crystals. *Anal.* ($C_8H_7ClO_4S$) Cl: calcd, 15.11; found, 14.73. The ir and nmr data are shown in Table I.

α-Sulfobenzylpenicillin (DL-I). A solution of DL-α-sulfophenylacetyl chloride (1.18 g, 5×10^{-3} mole) in 10 ml of dry ether was added dropwise to an ice-cooled, stirred solution of 6-APA (1.08 g, 5×10^{-3} mole) and $NaHCO_3$ (1.48 g, 1.76×10^{-2} mole) in water (8 ml). The mixture was allowed to react at 0–3° for 30 min. Then, the organic layer was separated, and the aqueous solution of the reaction mixture was collected and adjusted to pH 6.5. This was chromatographed on a column of Amberlite XAD-2 (100–200 mesh, 3.0 × 85 cm) with water as the eluent. The eluate was collected in 5-ml fractions in an automatically operated fraction collector under examination by uv absorption at 253.7 mμ. Two peaks appeared. The first small peak was due to α-sulfophenylacetic acid and was followed by the second main peak of the penicillin. Although there was partial overlapping with impurities derived from penicillin the appropriate fractionation made it possible to yield the penicillin free from inorganic salts, unreacted carboxylic acids, and several decomposition products from DL-I. The penicillin fraction, after being lyophilized, yielded a colorless powder: yield, 1.61 g. Recrystallization from ethanol gave colorless needles: 0.91 g, 38% from 6-APA; mp 269–274°; [α]D +171.8° (*c* 1.01, H_2O); ir (KBr disk) 3400 (OH), 2960 (CH), 1770 (C=O), 1675 (-CONH-), 1610 (COO^-), 1210 (SO_2), 1047; nmr (100 Mc, D_2O) δ 1.70, 1.76, 1.84 (3 sets of singlet, 6, $-CH_3$), 4.47, 4.51 (2 sets of singlet, 2, C_3-H), 5.33 (s, 1, side-chain methine), 5.74, 5.77, 5.80, 5.84 (4 sets of doublet, 2, all coupling constants = 4 cps, respectively), 7.6 (broad s, 5, Ph-H). *Anal.* ($C_{16}H_{16}O_7N_2S_2Na_2 \cdot H_2O$) C, H, N, S.

6-(α-Carboxy-α-phenylmethanesulfonamido)penicillanic Acid (VII, Disodium Salt). A solution of 432 mg (2.0×10^{-3} mole) of 6-APA and 692 mg (6.9×10^{-3} mole) of Et_3N in 15 ml of $CHCl_3$ was prepared and kept at room temperature. A solution of 547 mg (2.3×10^{-3} mole) of α-chlorosulfonylphenylacetic acid (V) in 7 ml of $CHCl_3$ was then added to the 6-APA solution. The resulting solution was stirred for 60 min at room temperature and refluxed for 4 hr. The reaction mixture, on evaporation, yielded a brownish residue which was dissolved in water (10 ml). After cooling and acidification to pH 2 with dil HCl, it was extracted with *n*-BuOH (10 ml × 2). The organic layer was separated and washed with 10 ml of cold H_2O. Water (20 ml) was added to the *n*-BuOH solution and the pH adjusted to 6.5 with saturated $NaHCO_3$ in H_2O. The H_2O layer was concentrated to a small volume (5 ml) *in vacuo*. This was chromatographed on a XAD-2 column (100–200 mesh, 3 × 10 cm) and was eluted with H_2O (160 ml) followed by 30% EtOH–H_2O (180 ml). The effluent was collected in 70-ml portions. The fraction containing XI, in lyophilization, gave 160 mg of a slightly yellow solid:

ir (KBr disk) 1770 (lactam C=O), 1675, 1610 ($-COO^-$) cm^{-1}; nmr (60 Mcps, D_2O) δ 1.55 (s, 3, CH_3), 1.67 (s, 3, CH_3), 4.29 (s, 1, C_3-H), 5.58 (s, 1, CH), 5.58 (2, C_5-H, C_6-H), 7.49 (5, Ph-H-). The MIC values in the *in vitro* tests are shown in Table II.

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Semisynthetic β-Lactam Antibiotics. 2.[1] Synthesis and Properties of D- and L-α-Sulfobenzylpenicillins[2]

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D(−)- and L(+)-α-sulfobenzylpenicillin (D- and L-I) were obtained by the following two methods: (1) acylation of 6-aminopenicillanic acid with D(−)- and L(+)-α-sulfophenylacetyl chloride and (2) separation of the diastereoisomeric mixture by means of column chromatography or by fractional crystallization from EtOH. D-I showed much more potent antibacterial activities than did L-I against *Pseudomonas aeruginosa* and other Gram-positive and Gram-negative organisms tested. A convenient nmr method was devised for estimating the optical purity of a partially isomerized mixture of D- and L-I. Stabilities of D-I, as measured in aqueous solutions, demonstrated that D-I is more stable than carbenicillin.

In the previous paper of this series[1] we reported the synthesis of a novel semisynthetic penicillin, *i.e.*, α-sulfobenzylpenicillin (DL-I), which showed potent antibacterial activity against a wide variety of Gram-positive and Gram-negative microorganisms including *Pseudomonas aeruginosa*. α-Sulfobenzylpenicillin, which has one asymmetric carbon in the acyl side chain, can exist as two diastereoisomers (D-I, L-I), which are expected to differ in antibacterial activity as in previous examples.[3] This paper deals with the syntheses of D- and L-α-sulfobenzylpenicillin and their physicochemical and biological properties.

DL-α-Sulfophenylacetic acid (DL-II) was successfully resolved by introducing basic natural amino acids into each enantiomorph (D-II, L-II) as described in the Experimental Section. The combination of sulfo and carboxyl group on the same carbon atom renders the α-hydrogen more acidic to the extent that each enantiomer is readily racemized in an alkaline solution. Addition of 1 equiv of NaOH to the enantiometric acid (D-II and L-II) yielded the optically active monosodium salt. However, the subsequent addition of another equivalent gave the disodium salt with complete loss of optical rotation. The absolute configuration assignment of each enantiomer (D-II, L-II) was accomplished on the monosodium salt of α-sulfophenylacetic acid by X-ray crystallographic studies in these laboratories.[4] Under conditions similar to those described in the preceding paper, the optically pure α-sulfophenylacetyl chloride (D-III, L-III) was synthesized by treatment of the optically pure acid with $SOCl_2$ without loss of optical rotation. After recrystallization from ether–hexane, completely resolved α-sulfophenylacetyl chloride, $[\alpha]D$ −23.7°, was obtained. The racemization of D- or L-III occurred in this reaction to some extent, the degree of racemization being smaller with a higher rate of reaction. When much solvent was used, the reaction time required for the conversion extended to a week or more at 30°, and the resulting product of III showed no optical rotation.

By treating 6-aminopenicillanic acid (6-APA) with D-III and L-III, each diastereomer of α-sulfobenzylpenicillin (D-I and L-I) was obtained with an optical purity of not less than 80%. Chromatography on XAD-2 was useful for purification of the reaction mixture and, moreover, for separation into each pure diastereoisomer. Thus, the L(+) epimer of the penicillin which showed a shorter retention time on the column was satisfactorily separated from the D(−) isomer. An alternative technique useful for resolving the diastereomeric mixture (DL-I) was fractional crystallization from ethanol. The colorless needles obtained proved to be the pure D(−) isomer, and the mother liquor contained L-I as the main component.

The 100-Mcps spectrum of D-I (D_2O, δ value) shows 2α- and 2β-methyl[5] protons at 1.70 and 1.76, H_3 at 4.47 (s), H_5 and H_6 at 5.74 (d, J = 4.0 cps) and 5.79 ppm (d, J = 4.0 cps), respectively, whereas the spectrum of L-I exhibits methyl protons at 1.76 and 1.84, H_3 at 4.51 (s), H_5 and H_6 at 5.80 (d, J = 4.0 cps) and 5.84 (d, J = 4.0 cps), respectively

The spectrum of DL-I shows clearly the presence of the two diastereoisomers, D-I and L-I (Figure 1). As for the protons attached to the acyl side chain, both D-I and L-I show equal signals at 5.33 (s) for H_{10} and at 7.5–7.9 (m, centered at 7.6) for phenyl protons. The assignment of the β-lactam ring protons was confirmed by the nmr spectra of the triethylamine salt in $CDCl_3$, which yielded a single-proton doublet centered at 5.58 and a single-proton quartet centered at 5.64 for H_5 and H_6, respectively. The H_6 quartet, existent in the lower field, consists of the coupling with the H_5 on the one side (J = 4.0 cps) and with the imino proton on the other (J = 10.5 cps). This is based on the fact that the quartet for H_6 collapsed to a doublet upon irradiation of the imino proton (δ 8.53), while irradiation of the peak at 5.58 changed the imino proton doublet (centered at 8.53) to a singlet.

The nmr spectra of D(−)- and L(+)-α-sulfobenzylpenicillin were significantly nonequivalent, presumably due to the dif-