Probiotics.† Antistaphylococcal and Antifibrinolytic Activities of ω-Amino- and ω-Guanidinoalkanesulfonic Acids[1,‡]

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A series of ω-aminoalkanesulfonic acids (1–5) and ω-guanidinoalkanesulfonic acids (6–9) has been tested for antistaphylococcal and antifibrinolytic activities. Most of the ω-aminoalkanesulfonic acids and 6 produced better protection against *Staphylococcus aureus* infections in mice than γ-aminobutyryl-L-histidine. Compound 4 was the best antistaphylococcal agent among the ω-aminoalkanesulfonic acids and compound 6 among the ω-guanidinoalkanesulfonic acids. Most of the ω-aminoalkanesulfonic acids have antifibrinolytic activity, while none of the ω-guanidinoalkanesulfonic acids has significant antifibrinolytic activity. Compound 4 possessed the highest antifibrinolytic activity which was equal to or greater than that of ε-aminohexanoic acid.

Earlier reports from this laboratory demonstrated that several series of compounds[2–6] were potent antistaphylococcal agents.

This communication deals with the antistaphylococcal and antifibrinolytic activities of ω-amino- and ω-guanidinoalkanesulfonic acids. To investigate a possible relationship between the biological response and physicochemical character, R_m values of ω-amino- and ω-guanidinoalkanesulfonic acids were also determined.

The compounds described in this paper are ω-aminoalkanesulfonic acids, $H_2N(CH_2)_nSO_3H$ [where n = 1, aminomethanesulfonic acid (1); n = 2, β-aminoethanesulfonic acid (2); n = 3, γ-aminopropanesulfonic acid (3); n = 4, δ-aminobutanesulfonic acid (4); n = 5, ε-aminopentanesulfonic acid (5)], and ω-guanidinoalkanesulfonic acids, $H_2NC(=NH)NH(CH_2)_nSO_3H$ [where n = 2, β-guanidinoethanesulfonic acid (6); n = 3, γ-guanidinopropanesulfonic acid (7); n = 4, δ-guanidinobutanesulfonic acid (8); n = 5, ε-guanidinopentanesulfonic acid (9)].

Chemistry. Compounds 2–5 were prepared by the method described by Marvel et al.[7] The dibromoalkane was treated with Na_2SO_3 in 95% EtOH under reflux. The resulting sodium ω-bromoalkanesulfonate was allowed to react with concentrated NH_4OH, yielding the ω-aminoalkanesulfonic acid. Since 1 could not be synthesized by this method, the method reported by Reinking et al.[8,9] was used successfully. The crude ω-aminoalkanesulfonic acid was purified by means of ion-exchange chromatography using AG 1 X8 anion-exchange resin, and H_2O and 0.02 *N* HCl as the effluent solutions. The cation-exchange resin, Amberlite CG-120, was used for further purification. Compounds 6–9 were prepared by a modification of the method described by Morrison et al.[10] Thus, a mixture of ω-aminoalkanesulfonic acids (1–5) and *S*-ethylisothiourea · H_2SO_4 in concentrated NH_4OH was heated to 60–75° and stirred vigorously until the reactants dissolved. There was a vigorous evolution of C_2H_5SH. The crude product was purified by ion-exchange chromatography. An attempt to prepare guanidinomethanesulfonic acid by the method of Morrison et al. failed because of the instability of 1; a modification of the method of Reinking et al.[8] also was unsuccessful.

R_m values were obtained by the method of Boyce and Milborrow.[11] Chromatography was carried out on glass plates (10 × 20 cm) coated with a 250-μ layer of silica gel G (E. Merck). After activation at 105° for 10 min, the plates were impregnated by allowing a 5% solution of paraffin oil white in hexane (v/v) to cover the plates; the solvent was evaporated at 40°. The mobile phase, $Me_2CO–H_2O$ (50:50, v/v), was allowed to run until the solvent front advanced 10 cm from the origin.

Yields, melting points, solvents, formulas, elementary analyses, and R_m values are shown in Table I. Table II shows R_f values of ω-amino- and ω-guanidinoalkanesulfonic acids on TLC. The compounds were homogeneous on TLC. Single spots were given by compounds 1–5 with ninhydrin, 6–9 with the Sakaguchi reaction, and all compounds with iodine and $K_2Cr_2O_7–H_2SO_4$.

Testing Procedure. In vivo antistaphylococcal activity was determined by the method described previously.[3–5] γ-Aminobutyryl-L-histidine was used as the positive control. Antistaphylococcal activity in vitro was determined in the same manner as before.[5] The lysis time and lysis area procedures for antifibrinolytic activity in vitro were described before[3–5] with ε-aminohexanoic acid as a positive control. Antistaphylococcal and antifibrinolytic activities of ω-amino- and ω-guanidinoalkanesulfonic acids are summarized in Table III.

Biological Results and Discussion. All the compounds in Table III (1–9 and γ-aminobutyryl-L-histidine) significantly protected (confidence level >97.5%, χ^2 test) mice against *Staphylococcus aureus* infection when compared with untreated infected mice (negative control). However, compound 4 was the only compound with significantly greater antistaphylococcal activity than γ-aminobutyryl-L-histidine at the 95% confidence level, although 3 was more effective at a confidence level >80%. To compare the effects of distance between functional groups and antistaphylococcal activity, the longest possible distance between N^ω of the amino or guanidino group and O'' of the acidic OH group was measured using CPK atomic models (Schwarz/Mann).[12] This distance was measured for the most active antistaphylococcal compound in each of the series which we have prepared, namely, δ-aminovaleric acid (ω-aminoalkanecarboxylic acids),[3] 4 (ω-aminoalkanesulfonic acids), δ-guanidinovaleric acid (ω-guanidinoalkanecarboxylic acids),[5] and 6 (ω-guanidinoalkanesulfonic acids). These distances are respectively 7.4, 7.7, 9.5, and 7.5 Å.

Most of the ω-aminoalkanesulfonic acids had antifibrinolytic activity while none of the ω-guanidinoalkanesulfonic acids displayed this activity. Activities (with ε-aminohexanoic acid as a positive control) are shown in Table III. Compound 4 was equal in activity to ε-aminohexanoic acid as confirmed by a comparison of different concentrations by the lysis time method (Figure 1). Previously, Okamoto[13a,b] found ε-aminohexanoic acid to be the most active antifibrinolytic agent among the ω-amino acids. It was reported[13c–e] that for maximum antifibrinolytic activity the longest possible distance between the terminal carbons of compounds with ω-amino and α-carboxyl functional groups was approximately 7 Å. The longest possible distance between N^ω of the amino group and O'' of the acidic OH

†The term probiotics has been proposed to designate these compounds and those described previously which build resistance to infection in the host but do not inhibit the growth of microorganisms in vitro.[5]

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Table I. ω-Amino- and ω-Guanidinoalkanesulfonic Acids

Compd	Yield, %[a]	Mp, °C[b]	Solvents	Formula	Analyses[c]	R_m[d]
1[e]	32.5	184–185 dec[f]	H_2O	CH_5NO_3S	C, H, N, S	−0.288
2[g]	61.8	264–266[h]	H_2O–EtOH	$C_2H_7NO_3S$	C, H, N; S[i]	−0.910
3[j]	81.0	269–271 dec[k]	H_2O–EtOH	$C_3H_9NO_3S$	H, S; C, N[l]	−0.956
4[m]	63.0	250–251 dec[n]	H_2O–EtOH	$C_4H_{11}NO_3S$	C, H, N, S	−0.956
5[o]	66.0	311–312[p]	H_2O–EtOH	$C_5H_{13}NO_3S$	C, H, N, S	−1.005
6[q]	48.0	266–267[r]	H_2O–EtOH	$C_3H_9N_3O_3S$	C, H, N, S	−0.956
7[s]	57.9	239–240	H_2O–EtOH	$C_4H_{11}N_3O_3S$	C, H, N, S	−0.940
8[t]	43.0	222–223	H_2O–EtOH	$C_5H_{13}N_3O_3S$	C, H, N, S	−0.796
9[u]	33.5	257–258	H_2O–EtOH	$C_6H_{15}N_3O_3S$	C, H, N, S	−0.657

[a]Based on sodium sulfite for **3-5**, ω-aminoalkanesulfonic acids for **6-9**, sodium β-bromoethanesulfonate for **2**, and sodium bisulfite for 1. [b]Melting points were determined by the capillary tube method and are uncorrected. [c]Analytical results for C, H, N, and S are within ±0.4% of the theoretical values except where indicated. [d]Silica gel G, 250-μ plates were used. Stationary and mobile phases were paraffin oil white and Me_2CO-H_2O (50:50, v/v), respectively. [e]Ir peaks (cm^{-1}) were 755, 894, 1010, 1060, 1082, 1186, 1240, 1321, 1460, 1515. [f]Lit.[8] 193°; lit.[9] 220° dec. [g]Ir peaks (cm^{-1}) were 740, 850, 896, 1040, 1050, 1117, 1120, 1188, 1218, 1256, 1312, 1350, 1397, 1435, 1467, 1518. [h]Lit.[9] 328°; Cortese[14] gives 300–305° (corrected); White and Fishman[15] give 327–328° dec. [i]S: calcd, 25.62; found, 26.20. [j]Ir peaks (cm^{-1}) were 592, 750, 790, 803, 836, 845, 938, 990, 1015, 1042, 1070, 1141, 1173, 1203, 1253, 1305, 1340, 1405, 1443, 1490. [k]Lit.[9] 290–292°; lit.[16] 292°. [l]C: calcd, 25.89; found, 25.08. N: calcd, 10.06; found, 9.47. [m]Ir peaks (cm^{-1}) were 597, 749, 794, 816, 852, 921, 1003, 1030, 1074, 1100, 1140, 1172, 1200, 1221, 1257, 1292, 1330, 1350, 1405, 1432, 1465. [n]Lit.[9] 263°; Helberger and Lantermann[17] give 222–223°; Derscherl et al.[18] give 260°; Weingarten[19] gives 270°. [o]Ir peaks (cm^{-1}) were 600, 727, 761, 783, 830, 917, 945, 1015, 1040, 1082, 1150, 1180, 1205, 1230, 1264, 1292, 1303, 1320, 1340, 1405. [p]Lit.[9] 310–312°; lit.[16] 310°. [q]Ir peaks (cm^{-1}) were 729, 744, 850, 970, 1000, 1030, 1075, 1155, 1192, 1250, 1340, 1405, 1460, 1620, 1680. [r]Lit.[10] 265°; Thoai and Robin[20] give 228–230°; Dittrich[21] gives 264°; Engel[22] gives 228°; Jinnai et al.[23] give 267°. [s]Ir peaks (cm^{-1}) were 700, 796, 834, 931, 1014, 1032, 1043, 1052, 1110, 1150, 1180, 1200, 1234, 1278, 1323, 1365, 1410, 1440, 1470, 1580, 1620, 1650, 1675. [t]Ir peaks (cm^{-1}) were 730, 784, 815, 945, 1038, 1047, 1069, 1115, 1162, 1180, 1222, 1254, 1280, 1305, 1325, 1355, 1405, 1420, 1445, 1470, 1610, 1630, 1675, 1680. [u]Ir peaks (cm^{-1}) were 700, 795, 831, 1010, 1035, 1070, 1078, 1110, 1156, 1196, 1215, 1235, 1274, 1283, 1305, 1350, 1370, 1400, 1430, 1460, 1560, 1590, 1630.

Table II. R_f Values of ω-Amino- and ω-Guanidinoalkanesulfonic Acids on TLC[a] ($R_f \times 100$)

Compd	Solvent 1	Solvent 2	Solvent 3	Solvent 4	Solvent 5
1	15	44	50	75	89
2	32	41	49	66	84
3	34	35	46	46	71
4	35	31	45	33	69
5	37	30	45	31	69
6	52	47	68	42	46
7	54	42	64	36	45
8	55	42	63	33	50
9	57	43	65	34	54
L-Ala[b]	35	41	46	44	80
L-Arg[c]	25	28	41	2	25

[a]Silica gel G (E. Merck), 250-μ plates (Analtech, Inc.) were used. [b]Positive control for compounds **1-5** with ninhydrin reaction. [c]Positive control for compounds **6-9** with Sakaguchi reaction. Solvent 1, PhOH-H_2O (75:25, w/v), pH 2.0; 2, n-BuOH-AcOH-H_2O (60:20:20), pH 2.4; 3, i-PrOH-formic acid-H_2O (77:4:19), pH 2.7; 4, sec-BuOH-MeOEt-dicyclohexylamine-H_2O (55:15:10:20), pH 10.3; 5, $CHCl_3$-MeOH-17% NH_4OH (40:40:20), pH 11.6.

group of **4**, as stated above, and of ε-aminohexanoic acid was 7.7 and 8.5 Å, respectively. The distance between N^{ω} and O″ of δ-guanidinovaleric acid, which had the highest antifibrinolytic activity among the ω-guanidinoalkanecarboxylic acids,[5] was 9.5 Å.

The results suggest that the maximum biological activity of both types depends upon the distance between the functional groups[3,5,13] and that the optimal distance, in turn, varies with the nature of the functional groups. Mangyo[13e] has suggested a relation of antifibrinolytic activity to the isoelectric point. A relationship between biological response and R_m values of ω-amino- and ω-guanidinoalkanesulfonic acids is also suggested in Figure 2. Since the sul-

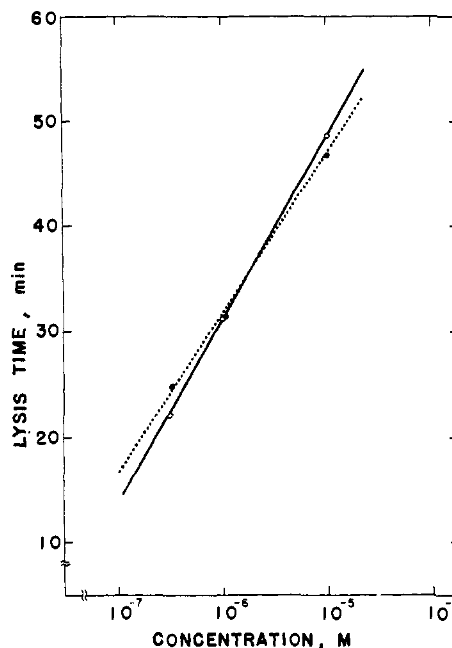

Figure 1. Antifibrinolytic activity of compound **4** and ε-aminohexanoic acid by the lysis time method: compound **4** (O——O); ε-aminohexanoic acid (●······●).

fonic acid function increases the solubility in aqueous phase, the R_m values of the two series of compounds were all in the minus region.

In summary, a series of ω-amino- and ω-guanidinoalkanesulfonic acids was found to have antistaphylococcal activity in vivo but did not inhibit the growth of *S. aureus* in vitro. Most of the ω-aminoalkanesulfonic acids were also antifibrinolytic agents. The activity of δ-aminobutanesulfonic acid was equal to that of ε-aminohexanoic acid by the lysis time method and greater by the lysis area method.

Table III. Antistaphylococcal and Antifibrinolytic Activities of ω-Amino- and ω-Guanidinoalkanesulfonic Acids

	Antistaphylococcal activity				Antifibrinolytic activity[b]			
			% protection[a]		Lysis time, min[c]		Lysis area, % inhibn[d]	
Compd	No. of mice	No. of trials	Mean	SD	Mean	SD	Mean	SD
1	25	3	32	20	9	3	1	4
2	25	3	45[e]	25	10	2	8	3
3	25	3	55	6	14	4	28	8
4	27	3	63	21	23	2	62	7
5	27	3	41	29	14[f]	3	21	7
6	24	3	43	8	8	2	3	2
7	24	3	33	9	9	2	4	2
8	24	3	38	16	9	4	4	3
9	24	3	38	20	8	2	2	1
γ-Abu-His[g]	46	6	35	19				
ε-Ahx[h]					22	4	42	12
Negative control[i]	50	6			7	1		

[a]Percent protection = [(mortality of control) − (mortality of experimental)] × 100/(mortality of control). [b]Sample concentration was 10^{-6} *M* in all experiments. [c]Number of minutes required for the clot to flow out from the test tubes. [d]Percent inhibition = [(lysis area of control) − (lysis area of experimental)] × 100/(lysis area of control). [e]Lit.[2] mean, 53%; SD, 3. [f]Okamoto[24] gives 7×10^{-5} *M* for minimal inhibitory concentration where ε-Ahx was 10^{-5} *M*; Okamoto et al.[13d] give 100 for inhibitory activity where ε-Ahx was 1000. [g]γ-Aminobutyryl-L-histidine was used as a positive control for antistaphylococcal experiments. [h]ε-Aminohexanoic acid was used as a positive control for antifibrinolytic activity tests. [i]Control for antifibrinolytic activity test, lysis time method, 0.05 *M* phosphate buffer–saline solution. The mortality of antistaphylococcal tests was 89%.

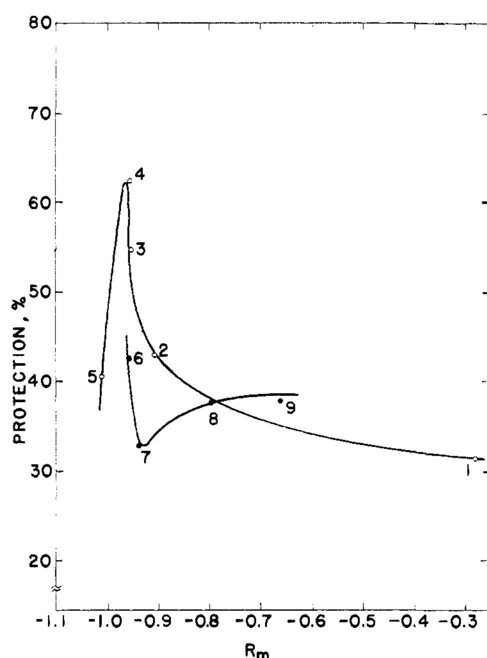

Figure 2. Relationship between the biological response and R_m values of ω-amino- and ω-guanidinoalkanesulfonic acids. Numbers refer to compounds: ω-aminoalkanesulfonic acids (O——O); ω-guanidinoalkanesulfonic acids (●——●).

Experimental Section

Sodium 2-bromoethanesulfonate, methyl bromide, 1,3-dibromopropane, 1,4-dibromobutane, and 1,5-dibromopentane were purchased from J. T. Baker Laboratory Chemicals and Products. *S*-Ethylisothiourea · H_2SO_4 was purchased from K&K Laboratories, Inc. Taurine (2) was purchased from Sigma Chemical Co. Anion-exchange resin, Bio-Rad AG 1 X8, was purchased from Bio-Rad Laboratories. Other chemicals and solvents were obtained from MC/B and Fisher Scientific Co. Melting points were taken by the capillary tube method and are uncorrected. Ir spectra (KBr) were taken with a Beckman infrared spectrophotometer, Model IR-20, for ω-aminoalkanesulfonic acids and with a Perkin-Elmer infrared spectrophotometer, Model 337, for ω-guanidinoalkanesulfonic acids. Elementary analyses were performed by Galbraith Laboratories, Inc.

Aminomethanesulfonic Acid (1). To a mixture of 111 g (1.06 mol) of $NaHSO_3$ in 139 ml of H_2O and 85.2 g (0.88 mol) of 36% CH_2O, 61.5 g of 28% NH_4OH was added dropwise through the separatory funnel at 75° with stirring. After the addition of NH_4OH, the mixture was allowed to stand at the same temperature for 1 hr and then cooled. The chilled reaction mixture was treated with concentrated H_2SO_4 (pH 1), obtaining fine, white crystals. After filtration, the crystals were washed with a small portion of cold H_2O and recrystallized from hot H_2O: yield 20.4 g (32.5%); mp 184–185° dec.

β-Aminoethanesulfonic Acid (Taurine 2). A solution of 11.0 g (0.052 mol) of $Br(CH_2)_2SO_3Na$ in 200 ml of concentrated NH_4OH was allowed to stand for a week at room temperature. The reaction mixture was then filtered and concentrated to dryness in vacuo. The crude product was purified using ion-exchange chromatography, AG 1 X8, 100–200 mesh, 2 × 40 cm column, OH^- form, 20 ml per fraction. The amounts of effluent solutions were 600 ml of H_2O and then 1 l. of 0.02 *N* HCl. The fractions of **2**, 46–54, were pooled and concentrated to dryness in vacuo and then crystallized from hot H_2O–EtOH: yield 5.2 g (61.8%); mp 264–266°. The ir spectrum was identical with that of a commercial sample of taurine.

γ-Aminopropanesulfonic Acid (3). In a 500-ml round-bottom flask fitted with a reflux condenser, a mechanical stirrer, and a separatory funnel were placed 40.4 g (0.2 mol) of $Br(CH_2)_3Br$, 76.5 ml of 95% EtOH, and 27.5 ml of H_2O. To the well-stirred boiling mixture, a solution of 8.32 g (0.066 mol) of anhydrous Na_2SO_3 in 32.5 ml of H_2O was added through the separatory funnel over a period of about 2 hr. The reaction mixture was then concentrated in vacuo. The $Br(CH_2)_3SO_3Na$ was extracted from NaBr and unreacted starting compounds with 150 ml of 95% EtOH, using a Soxhlet extractor. On cooling the solution, white crystals were obtained. The product was further purified by crystallizing from hot EtOH: yield 14.1 g (95.0%). The crude product was used for the next synthesis without further purification. Crude $Br(CH_2)_3SO_3Na$ (12.0 g, 0.053 mol) was treated with 200 ml of concentrated NH_4OH. The crude product was purified in the same way as **2**: yield 6.3 g (84.4%); mp 270–271° dec.

δ-Aminobutanesulfonic Acid (4). $Br(CH_2)_4Br$ (100.0 g, 0.46 mol) in 194 ml of 95% EtOH and 20 ml of H_2O was treated with

17.7 g (0.14 mol) of Na_2SO_3 in 60 ml of H_2O: yield 30.6 g (91.0%). Crude $Br(CH_2)_4SO_3Na$ (30.0 g, 0.13 mol) and 600 ml of concentrated NH_4OH were allowed to react in the same way as **2**: yield 13.3 g (69.0%); mp 251–252° dec.

ε-Aminopentanesulfonic Acid (5). $Br(CH_2)_5Br$ (100.0 g, 0.435 mol) in 200 ml of 95% EtOH and 60 ml of H_2O was treated with 16.8 g (0.133 mol) of Na_2SO_3 in 60 ml of H_2O: yield 29.9 g (89.0%). Crude $Br(CH_2)_5SO_3Na$ (22.0 g, 0.087 mol) was treated with 700 ml of concentrated NH_4OH in the same way as **2**: yield 10.8 g (74.0%); mp 311–312°.

β-Guanidinoethanesulfonic Acid (6). Into a solution of 6.3 g (0.05 mol) of **2** in 30 ml of concentrated NH_4OH, 10.1 g (0.05 mol) of *S*-ethylisothiourea · H_2SO_4 was added. The mixture was heated to 65° and stirred vigorously until the reactants dissolved. There was a vigorous evolution of C_2H_5SH and the mixture was allowed to cool to room temperature. The reaction mixture was concentrated to dryness in vacuo. The residue was dissolved in H_2O and insoluble unreacted *S*-ethylisothiourea was filtered off. The filtrate was then concentrated in vacuo to dryness. Ion-exchange chromatography with a 2.2 × 40 cm column of AG 1 X8 resin, 100–200 mesh, OH^- form, was used for the purification of **6**. NH_4OH (2 *N*), 0.5 *N* NH_4OH, and then H_2O were used successively as effluent solutions. Compound **2** was found in fractions 4–7. Fractions containing **6**, 10–70, were pooled and concentrated in vacuo. The dry residue was treated with H_2O–EtOH and kept at 5° to obtain fine, white crystals: yield 4.0 g (48.0%); mp 266–267°.

γ-Guanidinopropanesulfonic Acid (7). A mixture of 5.2 g (0.04 mol) of **3**, 8.1 g (0.04 mol) of *S*-ethylisothiourea · H_2SO_4, and 25 ml of concentrated NH_4OH was treated in the same manner as **6**: yield 4.2 g (57.9%); mp 239–240°.

δ-Guanidinobutanesulfonic Acid (8). A mixture of 1.53 g (0.01 mol) of **4**, 2.20 g (0.01 mol) of *S*-ethylisothiourea · H_2SO_4, and 10 ml of concentrated NH_4OH was treated in the same way as **6**: yield 0.84 g (43.0%); mp 222–223°.

ε-Guanidinopentanesulfonic Acid (9). A mixture of 5.0 g (0.03 mol) of **5**, 6.1 g (0.03 mol) of *S*-ethylisothiourea · H_2SO_4, and 25 ml of concentrated NH_4OH was allowed to react in the same manner as **6**: yield 2.1 g (33.5%); mp 257–258°.

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References and Notes

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Carbon-13 Magnetic Resonance Spectroscopy of Drugs. Sulfonamides

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The natural abundance ^{13}C magnetic resonance spectra of a series of sulfonamide drugs (sulfanilamide, sulfaguanidine, sulfathiazole, sulfasuxidine, sulfadiazine, sulfamerazine, sulfamethiazine, and sulfapyridine) have been determined at 25.15 MHz employing the pulse Fourier transform technique. The chemical shifts have been assigned with the aid of off-resonance and selective proton decoupling techniques, as well as by long-range carbon-13 proton coupling patterns.

Proton magnetic resonance (^1H NMR) spectroscopy has been used widely for structural studies of medicinally important molecules and for substrate–macromolecule binding studies.[1] Nevertheless, ^1H NMR spectra are often too complex to be helpful in the detailed structure analysis. This complexity results from the limited spectral range and from extensive spin–spin coupling. Therefore, the usefulness of ^1H NMR in structural studies of complicated mole-