SEMISYNTHETIC β-LACTAM ANTIBIOTICS. II[1]

PENICILLINS FROM α-HYDRAZINOARYLACETIC ACIDS

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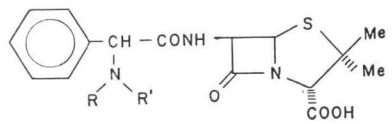
A number of penicillins (**2**) have been synthesized from the α-hydrazinoarylacetic acids (**4**) *via* the activated chloride hydrochlorides (**5**) or *via* the mixed anhydride of the corresponding N^2-benzyloxycarbonyl derivatives (**6**). The penicillins, **2b**, **e**, **j**, show good activity against gram-positive and gram-negative bacteria and enhanced penicillinase resistance in comparison with ampicillin.

Introduction of functional hydrophilic substituents, in particular an amino group, in the α-carbon atom of the side-chain of penicillin G markedly improves activity against gram-negative organisms.[2] Ampicillin (**1**), in fact, has become the most widely used broad spectrum antibiotic developed to date.

Despite various structural modifications performed, little information has been reported on analogues of **1** with basic nitrogen containing substituents[3] and, surprisingly, the amino group had not been replaced by the hydrazino residue.[4,5] We speculated that the more polar and bulky properties of a hydrazino group in comparison with the amino group of ampicillin could conceivably enhance the gram-negative spectrum and penicillinase resistance without loss of acid stability.



1: R = R' = H
2: R = NH_2, R' = H, Alkyl

Therefore, as a part of a systematic study on new oral semisynthetic β-lactam antibiotics, we undertook the synthesis and the antibacterial evaluation of new penicillins (**2**) derived from the α-hydrazinoarylacetic acids (**4**).

Chemistry

The syntheses of penicillins **2a** to **2l** are outlined in Scheme 1. The penicillins (**2**) were prepared according to method A starting from the α-hydrazinoarylacetic acids (**4**), which were recently described by us.[7] Activation of the carboxyl function was performed by treating **4**, protected as the hydrochloride, with phosphorous pentachloride in methylene chloride at low temperature. The acid chlorides **5a** to **5g** (Table 1) are white, crystalline, highly hygroscopic solids and are used as such for the next step. Condensation of **5** with 6-aminopenicillanic acid (6-APA) (**3**), protected as the trimethylsilyl ester, was performed in methylene chloride at low temperature in the presence of N,N-dimethylaniline (DMA). Subsequent mild hydrolysis gave the penicillins (**2**).

Alternatively, and only for 1-alkylhydrazino substituted penicillins, method B was used. The

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Scheme 1

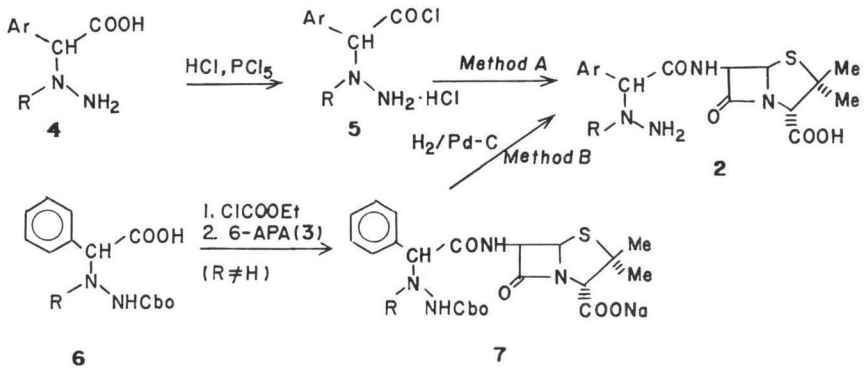
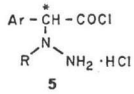


Table 1. α-Hydrazinoarylacetyl chloride hydrochlorides



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Compounds	Ar	R	Config at C*	Yield %	mp (°C dec)	νC=O ($cm^{-1}\pm5$)	Formula (a)
a	C_6H_5	H	*RS*	63	144~148	1770	$C_8H_{10}Cl_2N_2O$
	C_6H_5	H	*R*	66	145~148	1772, 1750	
	C_6H_5	H	*S*	51	142~144	1772, 1750	
b	C_6H_5	Me	*RS*	52	105~110	1785	$C_9H_{12}Cl_2N_2O$
	C_6H_5	Me	*R*	73	120~122	1790	(b)
	C_6H_5	Me	*S*	70	118~120	1790	
c	C_6H_5	Et	*RS*	49	80~ 85	1780, 1760	$C_{10}H_{14}Cl_2N_2O$
d	C_6H_5	*n*-Pr	*RS*	30	93~ 97	1795, 1770	$C_{11}H_{16}Cl_2N_2O$
e	2-Thienyl	H	*RS*	76	144~146	1770	$C_6H_8Cl_2N_2OS$
f	3-Thienyl	H	*RS*	50	158~159	1775	$C_6H_8Cl_2N_2OS$
g	2,5-Cl_2-3-thienyl	H	*RS*	73	158~160	1780	$C_6H_6Cl_4N_2OS$

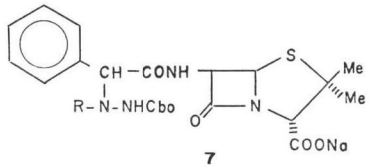
(a) except for **5b** *R*-form, all products were not obtained in analitically pure form and analyses (Cl, N) were within $\pm4\%$.

(b) Cl: calcd. 30.16–found 29.90; N: calcd. 11.91—found 12.03

sodium salts of 1-alkyl-2-benzyloxycarbonylhydrazinophenylacetic acids (**6**)[7] were treated with ethylchloroformate and the resulting mixed anhydride was condensed with an aqueous bicarbonate solution of 6-APA to give intermediates **7a** to **7d** (Table 2). These penicillins were easily isolated as sodium salts and are pure enough to be hydrogenated on 10% Pd-C in aqueous solution at room temperature and atmospheric pressure to give the corresponding penicillins **2d, g, h, i.**

Optical activity in the phenylacetic moiety was achieved for penicillins **2b, c,** and **2e, f** by starting from optically active **5a** (*R*), **5a** (*S*) and **5b** (*R*), **5b** (*S*) *via* method A. The *R*-epimers of these penicillins are highly crystalline, stable materials under the work up conditions; the *S*-epimers, on the contrary, are amorphous, more water soluble and labile both in solution and in the solid state. As a consequence, crystallization of penicillins **2a, j, k** and **l** (prepared from racemic **5a, e, f** and **g** respectively) from the reaction mixture took place with enrichment in the *R* epimer. The optical purity of these compounds

Table 2. 6-(α-2-Carbobenzyloxyhydrazinophenylacetamido)penicillanic acids sodium salts



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Compounds	R	Yield %	mp (°C dec)	ν C=O (cm^{-1} ±5)		Formula
				β-lactam	amide	
a	Me	58	165~173	1760	1665	$C_{25}H_{27}N_4NaO_6S$[a]
b	Et	71	168~170	1770	1670	$C_{26}H_{29}N_4NaO_6S$[b]
c	*n*-Pr	72	162~172	1770	1675	$C_{27}H_{31}N_4NaO_6S$[c]
d	*n*-Pent	54	167~178	1770	1675	$C_{29}H_{35}N_4NaO_6S$[c]

a) crystalline. *Anal.* Calcd.: N, 10.47; S, 5.99. Found: N, 10.08; S, 5.85.
b) crystalline. *Anal.* Calcd.: C, 56.92; H, 5.33; N, 10.21; S, 5.84.
Found: C, 56.52; H, 5.23; N. 9.81; S, 5.45.
c) amorphous solids with analyses (C, H, N, S) within ±5%.

was monitored by NMR spectroscopy (*e.g.* the chemical shifts of the *gem*-dimethyl groups are different in the two epimers).

The hydrazinopenicillins listed in Table 3 are generally microcrystalline zwitterions with high melting points and are poorly water soluble (with the exception of **2e**). The purity of **2a** to **2l**, established by IR[8], NMR, TLC and microanalyses, was greater than 90%.

Microbiology

The minimal inhibitory concentration (MIC) of the semisynthetic penicillins (**2**) was determined by the standard two-fold agar-dilution method. For all strains of bacteria, MIC was determined as the lowest concentration inhibiting bacterial growth. Each antibiotic was diluted appropriately in Brain Heart Infusion agar plus 10% horse serum and then poured into Petri dishes of 10 cm diameter. The plates were inoculated with overnight broth cultures, diluted 1/25 in Brain Heart Infusion broth using a multi-inoculating device[9] followed by incubation overnight at 37°C.

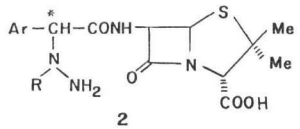
The acid stability of compounds **2** was evaluated by incubating them in 0.05 N HCl, pH 2, 37°C, for 60 minutes at a concentration of 500 μg/ml. The percentage of residual antimicrobial activity was then determined by microbiological assay after dilution in 1/15 M phosphate buffer, pH 6.

The resistance to penicillinase was assayed by incubating the compounds at a concentration of 500 μg/ml in 1/15 M phosphate buffer, pH 6, plus penicillinase Difco (approximately 10 U/ml) at 30°C for 60 minutes.

The MIC values of the penicillins **2a** to **2l** are compared with the MIC values of ampicillin (**1**), obtained under the same conditions, in Table 4.

It is evident that compounds **2** display an interesting antibacterial activity both against gram-positive and against some gram-negative strains. The most active penicillins in this series are **2b**, **e** and **j**, whose antibacterial activities are of the same order as that of ampicillin (**1**). Furthermore, all these compounds (**2**) are acid resistant, and are thus presumably suitable for oral administration. Some of these compounds (**2e, k, l**) are less sensitive than ampicillin to the action of penicillinase. This is also evident from their good *in vitro* activity against penicillinase producing strains.

Table 3. 6-(α-Hydrazinoarylacetamido)penicillanic acids



2

Compounds	Ar	R	Config at C*	Method	Yield %	mp (°C dec)	νC=O ($cm^{-1}\pm5$)		$[\alpha]_D^{20}$ [a] (deg)	Formula
							β-lactam	amide		
a	C_6H_5	H	*RS* [b]	A	53	217~220	1775	1695	+184.2	$C_{16}H_{20}N_4O_4S\cdot H_2O$ [e]
b	C_6H_5	H	*R*	A	50	190~195	1775	1695	+233.5	$C_{16}H_{20}N_4O_4S\cdot H_2O$ [d]
c	C_6H_5	H	*S*	A	61	187~190	1760	1670	+151.0	$C_{16}H_{20}N_4O_4S$ [e]
d	C_6H_5	Me	*RS*	B(A)	72 [f] (30)	212~217	1785	1680	—	$C_{17}H_{22}N_4O_4S$ [e]
e	C_6H_5	Me	*R*	A	30	176~178	1775	1690	+265.5 [g]	$C_{17}H_{22}N_4O_4S$ [h]
f	C_6H_5	Me	*S*	A	30	166~168	1785	1680	+204.5 [g]	$C_{17}H_{22}N_4O_4S$ [e]
g	C_6H_5	Et	*RS*	B	61 [f]	187~194	1770	1670	—	$C_{18}H_{24}N_4O_4S$ [e]
h	C_6H_5	*n*-Pr	*RS*	B	40 [f]	209~214	1770	1675	—	$C_{19}H_{26}N_4O_4S$ [e]
i	C_6H_5	*n*-Pent	*RS*	B	22 [f]	210~214	1770	1675	—	$C_{21}H_{30}N_4O_4S$ [e]
j	2-Thienyl	H	*RS* [b]	A	27	189~191	1780	1700	+218.5	$C_{14}H_{18}N_4O_4S_2$ [i]
k	3-Thienyl	H	*RS* [b]	A	37	190~191	1775	1690	+156.0	$C_{14}H_{18}N_4O_4S_2$ [i]
l	2,5-Cl_2-3-thienyl	H	*RS* [b]	A	30	184~185	1780	1695	+ 81.8 [k]	$C_{14}H_{16}Cl_2N_4O_4S_2$ [e]

[a] $c=0.05$; H_2O.

[b] The product contains the *R* isomer as the main component; **2a** was also characterized as *n*-butyl sulfamate salt [mp=106~108° dec; $[\alpha]_D^{20}$ +121.4° ($c=1$, MeOH)]. Anal. Calcd. for $C_{20}H_{31}N_5O_7S_2$: C, 46.41; H, 6.04; N, 13.53; S, 12.39. Found: C, 46.43: H, 6.40; N, 13.28; S, 12.00.

[c] *Anal.* Calcd.: C, 50.25; H, 5.80; N, 14.65; S, 8.38. Found: C, 50.57; H, 5.91; N, 14.61; S, 7.98.

[d] *Anal.* Calcd.: C, 50.23; H, 5.80; N, 14.65; S, 8.38. Found: C, 50.60; H, 5.90; N, 14.30; S, 8.00.

[e] Amorphous solid with analysis (C, H, N, S) within ±4%.

[f] From **6**.

[g] $c=0.5$; H_2O.

[h] *Anal.* Calcd.: C, 53.95; H, 5.86; N, 14.80; S, 8.47. Found: C, 53.60; H, 5.84; N, 14.40; S, 8.08.

[i] *Anal.* Calcd.: C, 43.29; H, 5.19; N, 14.42; S, 16.51. Found: C, 43.54; H, 5.08; N, 14.30; S, 16.13.

[j] *Anal.* Calcd.: C, 45.40; H, 4.90; N, 15.13; S, 17.31. Found: C, 42.95; H, 5.23; N, 13.50; S, 17.40.

[k] $c=0.05$; Me_2CO-H_2O 1: 1.

Table 4. Microbiological evaluation of penicillins **2**. Antibacterial activity (MIC in μg/ml)

Compounds	Gram-positive bacteria[a]					
	Staph. S.	Resistant *Staph.* P.	*Str. p.*	*Dipl. p.*	*B. subt.*	*S. lutea*
2 a	0.39	12.5	0.097	0.19	0.097	0.048
b	0.19	12.5	0.048	0.097	0.097	0.024
c	3.12	100	0.78	1.56	3.12	0.78
d	0.19	6.25	0.048	0.097	0.19	0.024
e	0.048	1.56	0.024	0.048	0.048	0.006
f	0.78	6.25	0.39	0.19	1.56	0.19
g	0.39	12.5	0.097	0.195	0.19	0.048
h	0.19	6.25	0.097	0.048	0.048	0.048
i	0.19	12.5	0.097	0.097	0.19	0.048
j	0.78	6.25	0.097	0.097	0.097	0.024
k	1.56	25	1.56	1.56	1.56	0.097
l	0.78	12.5	0.39	0.39	0.39	0.048
1	0.024	12.5	0.012	0.024	0.048	0.006

Compounds	Gram-negative bacteria[a]						Acid stability (% residual activity)	Penase stability (% residual activity)
	E. coli	*Sal. t.*	*Sh. d.*	*Pr. mir.*	*Pr. vul.*	*Ps. aer.*		
2 a	12.5	25	12.5	12.5	>200	>200	80.8	<20
b	3.12	12.5	3.12	6.25	200	>200	79.8	30
c	200	>200	200	200	>200	>200	—	—
d	6.25	25	12.5	12.5	100	200	77.5	21
e	3.12	6.25	6.25	12.5	25	100	100	61
f	50	200	100	200	200	200	95	<20
g	25	100	25	50	200	>200	59	25
h	50	100	50	100	200	>200	75	—
i	50	100	50	200	>200	>200	82	24
j	1.56	25	3.12	12.5	100	>200	83	<20
k	12.5	50	12.5	12.5	200	>200	100	50
l	12.5	50	6.25	50	>200	>200	100	40
1	0.78	3.12	0.78	3.12	200	>200	98	<20

[a] *Staph.* S.: *Staphylococcus aureus* Smith; *Staph.* P.: *Staph. aureus* PCI; *Str. p.*: *Streptococcus pyogenes* ISM 68/241; *Dipl. p.*: *Diplococcus pneumoniae* ISM 68/215; *B. subt.*: *Bacillus subtilis* ATCC 6633; *S. lutea*: *Sarcina lutea* ATCC 9341; *E. coli*: *Escherichia coli* 120; *Sal. t.*: *Salmonella typhimurium*; *Sh. d.*: *Shigella dyscenteriae* NCTC 4837; *Pr. mir.*: *Proteus mirabilis* ATCC 9921; *Pr. vul.*: *Proteus vulgaris* X20; *Ps. aer.*: *Pseudomonas aeruginosa* ATCC 9027.

The hydrazinopenicillins have therefore become the inspiration for a systematic synthetic program designed to optimize the microbiological activity.

Experimental Section

All melting points are uncorrected and were determined in open capillaries with a Büchi melting point apparatus. The optical rotation was determined at 20°C with a Perkin-Elmer 141 polarimeter. The IR spectra were obtained, unless stated otherwise, in Nujol mull with a Perkin-Elmer 157 spectro-

photometer and NMR spectra with a Perkin-Elmer R 12B spectrometer. In description of spectra the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet. Analytical results obtained for the products, were within ± 0.4% of the theoretical values, unless stated.

Preparation of α-hydrazinoarylacetic acids **4** and **6**

Racemic α-hydrazinoarylacetic acids **4** and the benzyloxycarbonyl derivatives **6** were prepared as previously described.[7] Resolution of α-hydrazinophenylacetic acid was accomplished according to DARAPSKY.[10]

R-α-(1-Methylhydrazino)phenylacetic acid (**4**, Ar=C_6H_5; R=Me)

Cooled dimethylformamide (50 ml, 0.65 mol) was allowed to react with thionyl chloride (43.7 ml, 0.61 mol) at 0°C for 10 minutes and diluted with CH_2Cl_2 (150 ml). The solution was cooled at −12°C and *R*-mandelic acid (30.4 g, 0.2 mol) was added portionwise. After 2.5 hours at 0°C, the reaction mixture was treated with crushed ice (250 g in two portions) and stirred for 15 minutes. The aqueous layer was separated and extracted with CH_2Cl_2 (100 ml). After washing with water and drying ($MgSO_4$), the solvent was evaporated *in vacuo*. The residue was taken up with CH_2Cl_2 (150 ml) and added dropwise to methylhydrazine (19 g, 0.41 mol) in CH_2Cl_2 (150 ml) at 0°C. After 1 hour the solution was evaporated *in vacuo*, EtOH (80 ml) was added and the pH was adjusted to 5.1 with 2 N HCl. The white solid was filtered and dried to give 21 g (60%) of **4**, mp 209~210°C.

IR (Fluorolube, cm^{-1}): maxima at 2900, 2600 and 2140 (νas and νs NH_3^+); 1620 (δas NH_3^+); 1575 (νas COO^-); 1530 (δs NH_3^+); 1385 (νs COO^-); 742 and 702 (γCH and φCC of monosubstituted phenyl ring).

$[\alpha]_D^{20}$ −141.5° (*c* 1, 1 N HCl)

Anal. ($C_9H_{12}N_2O_2$). Calcd.: C 59.78, H 6.71, N 15.54
Found: C 59.74, H 6.56, N 15.73.

The *S* epimer was obtained in the same way with *S*-mandelic acid; yield 50%, mp 193~194°C; $[\alpha]_D^{20}$ +138° (*c* 1, 1 N HCl).

Preparation of α-hydrazinoarylacetyl chloride hydrochlorides (**5a . . . g**)

General procedure:

Example: *R*-α-Methylhydrazinophenylacetyl chloride hydrochloride (**5b**)

Into a stirred suspension of *R*-α-1-methylhydrazinophenyl acetic acid (5 g, 28 mmol) in CH_2Cl_2 (50 ml) cooled at −60°C, dry hydrogen chloride was bubbled until saturation. Phosphorus pentachloride (8.4 g, 40 mmol) was added in one portion to the solution and the temperature was allowed to reach −30°C. After 30 minutes, crystallization was completed by stirring the suspension at 0°C for 30 minutes with CH_2Cl_2 (30 ml) to give 4.7 g of **5b** (Table 1).

IR (Fluorolube, cm^{-1}) maxima at 2900, 2700 and 2000 (νas and νs NH_3^+); 1790 (νC=O); 1605 (νC=C phenyl ring); 1570 and 1530 (δas and δs NH_3^+); 742 and 696 (γCH and φCC of monosubstituted phenyl ring).

6-(α-Hydrazinoarylacetamido)penicillanic acids (**2a . . . l**)

Method A

Example: 6-(*R*-α-Methylhydrazinophenylacetamido)penicillanic acid (**2l**)

To a suspension of 6-APA (1.5 g, 6.9 mmol) in CH_2Cl_2 (15 ml) and MeCN (15 ml), hexamethyldisilazane (HMDS) (1.45 ml, 6.9 mmol) was added and the mixture refluxed for 1.5 hours. The solution was evaporated *in vacuo*, the residue taken up with CH_2Cl_2 (15 ml), cooled at −15°C and DMA (0.96 ml, 7.6 mmol) was added dropwise. Then 1.7 g of **5b** (*R* form) (7.3 mmol) was added in four portions and the reaction mixture was allowed to reach 15°C in 1.5 hours. After cooling at −10°C, water was added and the aqueous layer was separated and filtered on a Celite pad. The pH was adjusted to 4.7 with triethylamine and *i*-PrOH (15 ml) and Et_2O (10 ml) were added. On standing at 10°C for 1 hour, 0.79 g of **2l** as white crystals were collected (Table 3).

IR (cm^{-1}): maxima at 3320 (νNH); 2600 and 2160 (νas and νs, NH_3^+); 1775 (νC=O, β-lactam); 1690 (νC=O amide); 1525 (δas NH_3^+); 1550 (νas COO^-).

NMR (in D_2O; reference standard DSS δ ppm): 7.55 s (5H; phenyl H); 5.51 s (2H; $C_{(5)}$ H and $C_{(6)}$ H); 4.88 s (1H; Ph-CH); 4.20 s (1H; $C_{(3)}$ H); 3.84 s (3H; CH_3–N); 1.45 s and 1.43 s (6H; *gem.* CH_3).

Method B

Example: 6-[*RS*-α-(2-Benzyloxycarbonyl-1-ethylhydrazino)phenylacetamido]penicillanic acid sodium salt (**7b**)

To a stirred suspension of α-(2-benzyloxycarbonyl-1-ethylhydrazino)phenylacetic acid sodium salt[5] (4.2 g, 12 mmol) in dry Me_2CO (90 ml) at −5°C were added 3 drops of 1% N-methylmorpholine in Me_2CO and then ethylchloroformate (1.48 g, 13.6 mmol). The suspension was stirred for 1.5 hours at room temperature, chilled and then poured into a solution of 6-APA (2.6 g, 12 mmol) in 4% $NaHCO_3$ (73 ml). After stirring for 30 minutes at 0°C and 40 minutes at room temperature, the solution was washed with Et_2O (2×50 ml). The aqueous solution was covered with Et_2O, the pH adjusted to 2 and the organic phase was extracted with 6.9% $NaHCO_3$ (100 ml). The aqueous solution was concentrated *in vacuo* at room temperature to give 4.67 g of **7b** (Table 2).

IR (Nujol; cm^{-1}): maxima at 3270 (νNH); 1770 (νC=O β-lactam); 1720 (νC=O carbobenzyloxy); 1670 (νC=O amide); 1605 (νas COO^-).

Anal. ($C_{26}H_{29}N_4NaO_6S$). Calcd.: C 56.92, H 5.33, N 10.21, S 5.84
Found C 56.52, H 5.23, N 9.81, S 5.45

6-[*RS*-α-(1-Ethylhydrazino)phenylacetamido]penicillanic acid (**2g**)

A stream of H_2 was passed through a stirred suspension of **7b** (3 g, 5.5 mmol) and 2.2 g 10% Pd-C in 30 ml of H_2O at 28°C for 1 hour until development of CO_2 ceased. The suspension was filtered, chilled and acidified to pH 2 with 2 N HCl. After washing with Et_2O the aqueous solution was adjusted to pH 4.7 and concentrated *in vacuo* at room temperature to give 1.85 (85.9%) of **2g** (Table 3).

IR (cm^{-1}): maxima at 3300 (νNH); 1760 (νC=O β-lactam); 1670 (νC=O amide); 1600 (νas COO^-).

NMR (in D_2O; reference standard DSS; δ ppm): 7.5 complex absorption (5H; phenyl H); 5.60 d (1/2 H; $C_{(5)}\underline{H}$ in one diastereoisomer; $J_{C_{(5)}\underline{H};\ C_{(6)}\underline{H}}$=4.0 Hz); 5.47 d (1/2 H; $C_{(6)}\underline{H}$ in one diastereoisomer; $J_{C_{(5)}\underline{H};\ C_{(6)}\underline{H}}$=4.0 Hz); 5.54 s (1H; $C_{(5)}\underline{H}$ and $C_{(6)}\underline{H}$ in the other diastereoisomer); 4.7 s (1H; Ph-$\underline{CH}$); 4.28 s and 4.26 s (1H; $C_{(3)}\underline{H}$ in the two diastereoisomers); 2.84 q (2H; $\underline{CH_2}$–N; $J_{CH_3;\ CH_2}$=7.0 Hz); 1.65 s, 1.62 s, 1.60 s and 1.55 s (6H; *gem* $\underline{CH_3}$ in the two diastereoisomers); 1.19 t (3H; $\underline{CH_3}$–CH_2–N; $J_{CH_3;\ CH_2}$=7.0 Hz).

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5) After this manuscript was completed it came to our attention that workers at Beecham in England had made compound **2a** and some analogous compounds by a different route[6]. This class of compounds was earlier described by us, B.P. 50944 (Nov. 2, 1973).
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