Study of the Structure of Besulpamide, 1-[(4-Chloro-3-sulfamoylbenzoyl)amino]2,4,6-trimethylpyridinium hydroxide inner salt, and related compounds, using X-Ray Crystallography and ^1H and ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The diuretic and antihypertensive drug Besulpamide, 1-[(4-Chloro-3-sulfamoylbenzoyl)amino]-2,4,6-trimethylpyridinium hydroxide inner salt, and related compounds have been investigated by nmr spectroscopy and mass spectrometry. A mechanism for the formation of the salt **5** is proposed. The tautomerism of hydroxy derivatives of Besulpamide is discussed on the basis of nmr spectroscopy. The single-crystal X-ray investigation of Besulpamide, R = 0.038 (R_w = 0.041), showed two crystallographically independent molecular conformations in the crystal structure, space group $P\bar{1}$, a = 8.485(3), b = 14.282(2), c = 15.312(6), α = 69.41(2), β = 82.22(4), γ = 72.78(3).

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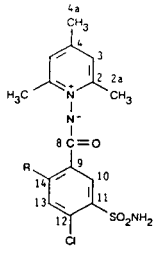
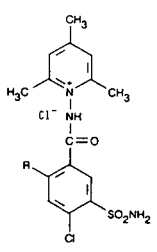
4-Chloro-3-sulfamoylbenzoyliminopyridinium betaines, which exhibited both diuretic and antihypertensive activities, have been synthesised in our laboratory [1]. The 1-[(4-chloro-3-sulfamoylbenzoyl)amino]-2,4,6-trimethylpyridinium hydroxide inner salt, Besulpamide (**1**), has been chosen for further development, and is at present in phase II clinical trials [2].

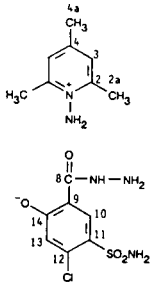
The pharmacokinetics of Besulpamide have been studied in rats and dogs [3], but its metabolism is not as yet completely clarified. In order to identify some metabolites, the hydroxy derivative of Besulpamide **2** was synthesised by the standard procedure [1,4] of refluxing 4-chloro-2-hydroxy-5-sulfamoylbenzoylhydrazide, **6**, with trimethylpyrilium tetrafluoroborate and subsequent treatment with potassium hydroxide. By this reaction another well defined crystalline compound was isolated and identified as the salt **5**.



1 (R = H)
2 (R = OH)



3 (R = H)
4 (R = OH)



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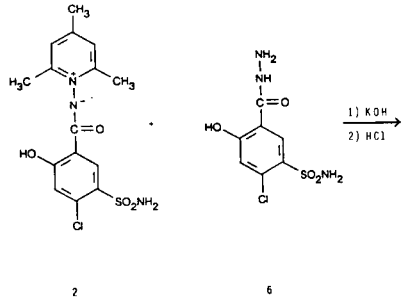
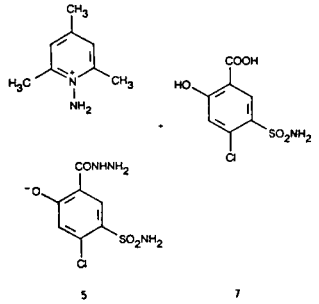
Extensive studies carried out by Katritzky *et al.* [5] concerning the nucleophilic attack on *N*-substituted 2,4,6-triarylpyridinium salts allow the conclusion that the *N*-substituents can be displaced and that the pyridine ring can act as the leaving group. This research group also showed [4a,5] that the pyrolysis of the *N*-acylamines of 2,4,6-triarylpyridinium salts leads to fragmentation of the N-N bond to give excellent yields of the corresponding isocyanates. In our case, the formation of compound **5** involves the breaking of the N-CO bond.

The proposed mechanism for the formation of the salt **5** is outlined in Scheme I. It involves the reaction of the previously formed betaine **2** with the hydrazide **6** and potassium hydroxide. In order to confirm this hypothesis **2** and **6** were refluxed in methanol. Our initial experiments were conducted with one equivalent of potassium hydroxide in ethanol or in methanol as solvent but only unreacted compounds were recovered. The addition of water also led to unchanged compounds. In contrast, when 2.2 equivalents of potassium hydroxide were used, the salt **5** was recovered by crystallization and, after acidification of the filtrate, 4-chloro-2-hydroxy-5-sulfamoylbenzoic acid, **7**, was identified by ^1H-nmr.

Scheme I



2 6



5 7

Table 1

^{1}H NMR Data of Compounds **1-5** [a]

Hydrogen	1	1 [b]	2	3	4	5
H(3)	7.60 (s)	7.25 (s)	7.62 (s)	7.94 (s)	7.94 (s)	7.67 (s)
H(2a)	2.50 (s)	2.58 (s)	2.52 (s)	2.68 (s)	2.61 (s)	2.70 (s)
H(4a)	2.50 (s)	2.48 (s)	2.52 (s)	2.59 (s)	2.61 (s)	2.43 (s)
H(10)	8.77 (d) [c]	8.89 (d) [c]	8.59 (d) [e]	8.71 (d) [c]	8.46 (s)	8.23 (d) [f]
H(13)	7.66 (d) [d]	7.55 (d) [d]	7.08 (d) [e]	7.87 (d) [d]	7.63 (s)	6.32 (d) [f]
H(14)	8.25 (dd) [c,d]	8.25 (dd) [c,d]	-	8.55 (dd) [c,d]	-	-
SO_2NH_2	7.63 (s)	7.26 (s)	7.41 (b)	7.70 (b)	7.82 (b)	7.05 (b)
N*H*	-	-	-	5.23 (b)	7.82 (b)	12.05 (b)
O*H*	-	-	14.38 (b)	-	11.83 (b)	-
Other H	-	-	-	-	-	4.14 (b, NH_2)
						6.85 (b, NH_2)

[a] Solvent: hexadeuteriodimethyl sulfoxide if not otherwise indicated. TMS was used as internal standard. [b] Solvent: deuteriochloroform and TMS was used as internal standard. Coupling constants in hertz. [c] $J_{10,14} = 2$. [d] $J_{13,14} = 8$. [e] $J_{10,13} = 1.5$. [f] $J_{10,13} = 1.2$.

Table 2

^{13}C NMR Data of Compounds **1-5** [a]

Carbon	1	2	3	4	5
C(2)	152.2	152.2	156.2	155.9	153.5
C(3)	126.1 [c]	126.2 [c]	128.0 [c]	127.7 [c]	127.1 [c]
C(4)	150.4	151.9	160.1	159.2	154.2
C(2a)	18.8	18.6	19.0	19.2	19.7
C(4a)	20.5	20.3	21.6	21.6	20.1
C(8)	165.5 [e,f]	167.7 [b,e]	163.0	163.0	166.5 [b,h]
C(9)	137.1 [b,l]	116.7 [b,f,n]	129.4 [b,d]	115.5 [d]	116.1 [b,f,g]
C(10)	128.4 [c,i]	130.3 [c]	129.1 [d]	132.2 [c,d]	131.6 [c]
C(11)	140.5 [b,h]	130.6 [g,h]	141.8 [b]	132.1 [d]	119.6 [g,i]
C(12)	131.5 [b,i,l]	133.2 [b,e]	135.5 [b]	135.6 [b]	132.3 [b,j,k]
C(13)	131.0 [c]	118.8 [c,m]	132.2 [c]	119.5 [d]	123.1 [c]
C(14)	132.2 [c,i]	163.3 [b,h,n]	132.6 [c]	160.8 [b]	173.5 [b,g,l]

[a] Chemical shifts measured in ppm from the central line of DMSO-d_6 and corrected to TMS using and offset of 39.7 ppm. [b] Assignment confirmed by HETNOE enhancement (see Table 3). [c] Assignment confirmed by intermediate power selective decoupling of directly attached protons. [d] Assignment confirmed by SEFT. Coupling constants in hertz: [e] $^3J_{CH} = 4$. [f] $^3J_{CH} = 5$. [g] $^2J_{CH} = 1.5$. [h] $^3J_{CH} = 6$. [i] $^3J_{CH} = 7$. [j] $^3J_{CH} = 2$. [k] $^3J_{CH} = 10$. [l] $^3J_{CH} = 8$. [m] The couplet spectrum shows a doublet ($^1J_{CH} = 167$ hertz) with further long range coupling. [n] The couplet spectrum shows a doublet with further long range coupling.

In *N*-acylimines of pyridinium salts, the negative charge can be delocalized in the exocyclic substituent [6a] (Scheme II, mesomeric structures **2a** and **2b**). The possibility of tautomers of compound **2** must also be considered. Particularly, tautomer **2c** and **2f** are especially favorable for breakage of the N-CO bond.

The structural study of the betaines **1** and **2**, their hydroclorides **3** and **4** and the salt **5** has been achieved using

^1H and ^{13}C nmr spectroscopy and mass spectrometry. A single crystal X-ray structure analysis of Besulpamide, **1**, was also undertaken.

Nuclear Magnetic Resonance Spectroscopy.

The ^1H and ^{13}C nmr chemical shifts and coupling constants of compounds **1-5** are shown in Tables 1 and 2. The numbering of all carbon atoms in the Tables is the same as that adopted in the X-ray crystallographic structure of Besulpamide (Figure 2) in order to provide direct comparison.

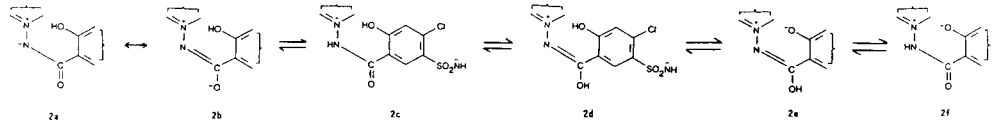
^1H nmr of a series of *N*-acyliminepyridinium have been reported [6] as well as systematic comparative studies in *N*-substituted pyridines [7]. The assignment of protons of compounds **1-5** is straightforward. The chemical shifts of α- and γ-methyl protons of the pyridinium ring are the same in DMSO-d_6 for the compounds **1, 2** and **4** but the γ-methyl group is less deshielded than α-methyl in compounds **3** and **5** and in compound **1** using deuteriochloroform as solvent. The same was observed earlier [8a] with non-aromatic *N*-bonded trimethylpyridinium salts but the situation is reversed when *N*-aryl substituents are present. As in compound **1** using deuteriochloroform as solvent, the *N*-benzenesulfonylimino group shields the γ-methyl protons and deshields the α-methyl protons [8b].

Comparison of the chemical shifts of compounds **1** and **2** shows that hydroxy substitution at C(14) produces an upfield shifts (−0.58) [9] in the H(13) and in the H(10) (−0.18). These data exhibit a considerable parallelism (−0.56 and −0.12 respectively) with those described in the literature [10] for the hydroxy substitution, suggesting that the hydroxy tautomers **2a** to **2d** (Scheme II) are the predominant forms. The H(13) and H(10) in compound **5** are shielded (−0.76 and −0.36 respectively) in comparison with the same protons in compound **2** due to the major contribution of phenolate tautomers **5e** and **5f** (Scheme III). The data reported in the literature [11] show that, on conversion to the anion, the resonance of the *ortho* and *meta* protons of a substituted phenol are shifted upfield (−0.50 to −0.84 and −0.19 to −0.47 respectively).

The ^{13}C nmr spectra of compounds **1-5** are given in Table 2. The resulting chemical shifts of **1** agree reasonably well with those reported earlier [12] for unsubstituted 1-benzoylaminopyridinium hydroxide, inner salt. However, the identification and assignments of the ^{13}C shifts of compound **5** are essential to the establishment of its constitution, as well as for compounds **1-5** in order to discuss their tautomerism. The signals of all protonated carbons were unambiguously assigned by intermediate power selective decoupling of the corresponding protons (SFORD technique [13a]) and by the spin echo Fourier transform sequence (SEFT [13b]). These techniques permitted assignment of the narrow range of resonances at 131.0 and 131.5 ppm for compound **1**, at 130.3 and 130.6 ppm for compound **2**, at 132.2 and 132.6 ppm for compound **3**, at 132.1 and 132.2 ppm for compound **4** and at 131.6 and 132.3 ppm for compound **5**. Some quaternary carbons were assigned by examination of the couplet spectra. Thus, the characteristic coupling constants C-H for compound **1** allow assignment of carbons C(8) and C(12) and the broad doublets for compound **2** of carbons C(9) and C(14) may indicate their position with respect to the OH proton. A broad coupling is also observed at C(13) of compound **2** but couplings observed at carbons C(9), C(13) and C(14) for compound **5** were not broad, showing the absence of OH appended at C(14).

However, the assignments of the C(9) and the C(11) carbons fo all compounds, and of the C(8) and the C(14) carbons of compounds **2** and **5** remained uncertain. We used the HETNOE technique, developed by Sanchez-Ferrando *et al.* [14], which we have recently applied to confirm the constitution of some quinolone derivatives [15a] and to the constitutional assignment of the antiinflammatory Droxicam [15b]. A series of HETNOE experiments (Table 3) provided conclusive evidence of the assignments of the quaternary carbons and explained the constitution and the tautomeric predominant forms of the betaine **2** and of the salt **5**. The HETNOE method, as shown previously [16], is very useful in hydrogen bonded systems. In our cases, the crucial HETNOE experiments were caried out by low power presaturation of the OH signals at δ 14.38

Scheme II



Scheme III

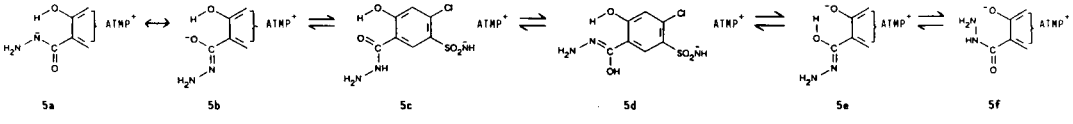


Table 3

Percentage Heteronuclear NOE Enhancements on Irradiation of some Protons in DMSO-d_6

Compound	Irradiated Proton	NOE % C(8)	C(9)	C(11)	C(12)	C(14)
1	H(10)	24	65	92	-	-
	H(13)	-	-	-	62	-
	H(14)	-	17	-	-	-
2	H(13)	-	-	-	84	18
	O*H* [a]	37	30	-	-	55
3	H(10)	23	68	93	-	-
	H(13)	-	-	-	65	-
4	H(13)	-	-	-	49	19
5	H(13)	-	-	-	75	60
	O*H* [a]	29	24	-	-	8

[a] Irradiation time to generate nOe on quaternary carbons = 180s.

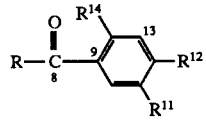
and 12.05 ppm of compounds **2** and **5** respectively, for an irradiation time to generate nOe on quaternary carbons of 180 s. This resulted in enhancements, for the betaine **2**, of 55% at C(14), 37% at C(8) and 30% at C(9), thus showing the intramolecular hydrogen bond and therefore the predominant tautomeric structures **2a** and **2c** (Scheme II). The irradiation of the OH signal of the salt **5** resulted mainly in nOe enhancement (29%) at C(8) and only 8% at C(14), thus showing that the negative charge is located at the oxygen attached at C(14) and therefore the predominant tautomeric structures are **5e** and **5f** (Scheme III).

The C(2) and C(4) carbons of the pyridine ring underwent a shift to low field on protonation (compounds **3** and **4**) of the benzamidate nitrogen atom of the betaines **1** and **2**. In contrast, the carbonyl carbons C(8) and C(9) become more shielded on protonation on going from compound **1** to **3** and from compound **2** to **4**.

A specific problem was raised on considering the effect of hydroxy substitution to the carbonyl group. For the betaines **1** and **2**, the change of an OH causes a downfield shift (+2.2 ppm) [17] at the carbonyl carbon C(8) and an upfield shift (−20.4 ppm) at C(9). For the protonated compounds **3** and **4**, no change in the chemical shift is produced at C(8) and an upfield shift of −13.9 ppm is observed at C(9). We interpret these observations as being consistent with the view that intramolecular hydrogen bonding is present in the betaine **2** and do not exist in the protonated compound **4**. Indeed, it has been described [13c] that intramolecular hydrogen bonding involves the nonbonding electron of the carbonyl oxygen and, as a result, the carbonyl carbon becomes more positive, and thus a deshielding is observed. On the other hand, the experimentally determined ^{13}C nmr spectra of several compounds with intramolecular hydrogen bonding (Table 4) showed that not only the carbonyl carbon C(8) is more deshielded

Table 4

Substituent Increments in Substituted Benzenes [a]



R	R^{11}	R^{12}	R^{14}	Δδ C(8)	Δδ C(9)	Δδ C(13)
OH	SO_2NH_2	Cl	H-->OH	+ 4.6	- 18.0	- 13.8
OCH_3	SO_2NH_2	Cl	H-->OH	+ 2.4	- 16.4	- 13.3
OCH_3	SO_2NHMe	Cl	H-->OCH_3	- 0.7	- 10.6	- 17.4
OCH_3	SO_2NMe_2	Cl	H-->OCH_3	- 0.3	- 10.5	- 17.5
NH_2	SO_2NH_2	Cl	H-->OH	+ 2.9	- 19.1	- 12.1
NH_2	SO_2NMe_2	Cl	H-->OCH_3	- 1.7	- 11.8	- 16.7
$NHNH_2$	SO_2NH_2	Cl	H-->OH	+ 2.5	- 16.2	- 8.5
NH_2	H	H	H-->OH	+ 3.5	- 19.6	- 10.9
NH_2	H	H	H-->OCH_3	- 2.1	- 11.8	- 16.2

[a] See footnote [a] on Table 2.

(+2.4 to +4.6) but also the α-carbon C(9), with respect to the OH and the carbonyl groups, is shielded by −16.2 to −19.6 ppm. In contrast, when intramolecular hydrogen bonding was not possible (OCH_3 as substituent in Table 4), the carbonyl carbon C(8) underwent a shift to high field (−0.3 to −2.1) and the α-carbon C(9) was shielded only by −10.5 to −11.8 ppm, taking into account that the shielding effect of a methoxy group is higher than that of an hydroxy substituent. In fact, the C(13) carbon is shielded between −8.5 and −13.8 ppm by an OH and between −16.2 and −17.5 ppm by a methoxy group (Table 4). However, the hydroxy effect at C(13) carbon in the betaine **2** (−12.2 ppm) is nearly equal to that of the protonated compound **4** (−12.7 ppm).

In conclusion, the predominant tautomeric structures of the hydroxy derivatives of Besulpamide are **2a** and **2c** (Scheme II), **5e** and **5f** (Scheme III) and **4b**.

Mass Spectrometry.

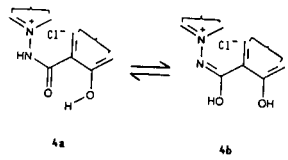
The electron impact spectrum of Besulpamide **1** (Table 5), revealed a fragmentation pattern analogous to the *N*-benzoylimino-2,6-dimethylpyridinium betaine, studied by Ikeda *et al.* [18]. The spectrum showed the molecular ion (m/z 353) and the base peak at m/z 338 corresponding to a loss of an α-methyl radical and subsequent cyclization of the oxygen radical to give a fragment ion of completely aromatized structure (Figure 1).

Table 5

Mass Spectrometric Fragmentation [a]

m/z	1	2	5	6
369		3		
354		5		
353	4			
338	100			
265			5	21
234		4	33	100
218	13			
163	30	7		
137			9	
136	39	32	78	
121	45	38	100	
120	20	39	81	

[a] Intensity of some of the more important fragments expressed as percent of base peak.



4a 4b

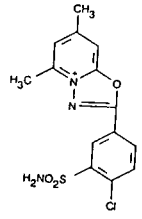


Figure 1

A loss of 4-chloro-3-sulfamoylphenyl from molecular ion led to m/z 163 which by further elimination of NCO furnished a trimethylpyridinium ion at m/z 121. A low intensity peak at m/z 218 corresponds to the 4-chloro-3-sulfamoylbenzoyl ion as described for related compounds [19].

The fragmentation pattern of compound **2** is analogous to that of **1**. The salt **5** shows peaks at m/z 265 and 234 corresponding to 4-chloro-2-hydroxy-5-sulfamoylbenzoylhydrazide and those corresponding to the fragmentation of trimethylpyridinium (m/z 136, 121 and 120). Moreover, the peek of *N*-trimethylpyridiniumisocyanate at m/z 163 is not observed in the spectrum of compound **5** and the cation of the salt **5** at m/z 137 is not observed in the spectra of compounds **1** and **2** (Table 5).

The X-Ray Crystallographic Description of Besulpamide, **1**.

The structure determination was carried out as described in the experimental section. Two independent molecular conformations (**1** and **1'**) were identified in the crystals as shown in Figure 2. The final fractional atomic co-ordinates and bond lengths involving H-atoms are given in Table 6, and bond lengths and bond angles in Table 7.

The atoms in the pyridinium ring are coplanar to within ± 0.018 Å in molecules **1** and **1'**, while those for the benzene ring are within ± 0.032 Å in molecule **1** and ± 0.041 Å in molecule **1'**. The atoms in the $\overset{+}{N}\text{-}\overset{-}{N}$-CO-C(9) system are also coplanar to within ± 0.009 Å in molecule **1** and ± 0.012 Å in molecule **1'**. The other parameters of interest in molecules **1** and **1'** are the dihedral angles between the least-squares ring planes and the $\overset{+}{N}\text{-}\overset{-}{N}$-CO-C(9) system and the torsion angles of this part of the molecule. The pyridinium ring is almost perpendicular to the $\overset{+}{N}\text{-}\overset{-}{N}$-CO-C(9) system (85.1° for molecule **1** and 96° for molecule **1'**), as required by space group symmetry. The dihedral angles between the $\overset{+}{N}\text{-}\overset{-}{N}$-CO-C(9) system and the benzene

Table 6

Fractional Atomic Coordinates (x 10^4) and Bond Distances (Å) Involving H-Atoms with their e.s.d.'s

Molecule 1				Molecule 1'			
Atom	x	y	z	Atom	x	y	z
N1	7208(4)	5711(2)	8548(2)	N1'	13340(3)	1397(2)	14908(2)
C2	5710(5)	6077(3)	8926(3)	C2'	13941(4)	2189(2)	14877(2)
C2a	4216(7)	5920(5)	8643(4)	C2a'	14686(5)	2751(3)	13980(3)
C3	5637(6)	6582(3)	9558(3)	C3'	13789(4)	2457(2)	15675(2)
C4	7029(6)	6705(3)	9819(3)	C4'	12993(4)	1966(3)	16474(2)
C4a	6924(9)	7204(4)	10550(4)	C4a'	12724(8)	2319(4)	17319(3)
C5	8508(6)	6348(3)	9393(3)	C5'	12414(4)	1166(3)	16471(2)
C6	8615(5)	5857(3)	8750(3)	C6'	12604(3)	864(2)	15691(2)
C6a	10175(6)	5499(5)	8238(4)	C6a'	12049(5)	-26(3)	15663(3)
N7	7278(4)	5212(2)	7886(2)	N7'	13570(3)	1084(2)	14109(2)
C8	7627(5)	4195(3)	8270(3)	C8'	12254(4)	1519(2)	13593(2)
O8a	7893(4)	3679(2)	9118(2)	O8a'	10920(3)	2122(2)	13734(1)
C9	7668(5)	3648(3)	7585(3)	C9'	12453(3)	1181(2)	12744(2)
C10	7813(5)	4140(3)	6628(3)	C10'	11043(4)	1272(2)	12320(2)
C11	7920(4)	3622(3)	5993(3)	C11'	11170(3)	901(2)	11576(2)
C12	7879(5)	2588(3)	6331(3)	C12'	12726(4)	461(2)	11252(2)
CL12	8121(1)	1861(1)	5600(1)	CL12'	12947(1)	-70(1)	10366(1)
C13	7700(5)	2097(3)	7282(3)	C13'	14145(4)	407(3)	11648(2)
C14	7592(5)	2632(3)	7899(3)	C14'	13997(4)	766(3)	12393(2)
S15	8140(1)	4305(1)	4779(1)	S15'	9307(1)	957(1)	11117(1)
O15a	8112(4)	5331(2)	4723(2)	O15a'	9332(3)	-94(2)	11258(1)
O15b	6943(3)	4174(2)	4294(2)	O15b'	7995(3)	1564(2)	11538(2)
N16	9932(6)	3790(3)	4427(3)	N16'	9360(4)	1556(2)	10023(2)
H2a1	4341(63)	5239(42)	8744(36)	H2a1'	13851(51)	3081(30)	3546(28)
H2a2	3271(68)	6083(39)	8997(35)	H2a2'	15457(51)	2304(32)	3764(28)
H2a3	4064(66)	6173(41)	8064(38)	H2a3'	15213(49)	3218(32)	4081(27)
H3	4626(64)	6790(38)	9780(35)	H3'	14260(47)	2979(30)	5651(27)
H4a1	6407(61)	6763(39)	11140(38)	H4a1'	12733(56)	2916(34)	7180(31)
H4a2	7845(66)	7374(40)	10631(36)	H4a2'	13955(51)	2094(29)	7668(28)
H4a3	6197(64)	7758(41)	10508(36)	H4a3'	12388(52)	1891(32)	7842(30)
H5	9455(65)	6471(38)	9441(34)	H5'	11830(49)	850(30)	6961(28)
H6a1	10342(61)	4751(42)	8355(35)	H6a1'	12864(50)	-486(31)	5459(27)
H6a2	11044(66)	5591(40)	8471(36)	H6a2'	11584(49)	-378(31)	6281(29)
H6a3	10168(61)	5845(39)	7633(38)	H6a3'	11132(52)	215(30)	5288(28)
H10	7954(61)	4840(40)	6436(35)	H10'	9931(48)	1567(29)	2533(26)
H13	7563(58)	1370(40)	7490(33)	H13'	15180(50)	139(29)	1377(27)
H14	7540(59)	2265(38)	8560(36)	H14'	14982(49)	725(29)	2644(26)
H161	10100(57)	3132(37)	4290(32)	H161'	9827(50)	1193(32)	-293(28)
H162	10769(60)	3930(37)	4672(33)	H162'	8879(51)	2190(33)	-158(28)

Bond	Length	Bond	Length
H2a1 ---C2a	0.905 (61)	H2a1'---C2a'	0.935 (41)
H2a2 ---C2a	0.923 (52)	H2a2'---C2a'	0.887 (42)
H2a3 ---C2a	0.843 (55)	H2a3'---C2a'	0.968 (53)
H3 ---C3	0.883 (50)	H3' ---C3'	0.934 (49)
H4a1 ---C4a	1.030 (50)	H4a1'---C4a'	0.804 (49)
H4a2 ---C4a	0.919 (65)	H4a2'---C4a'	1.145 (45)
H4a3 ---C4a	0.835 (50)	H4a3'---C4a'	0.890 (40)
H5 ---C5	0.942 (63)	H5' ---C5'	0.898 (38)
H6a1 ---C6a	0.987 (60)	H6a1'---C6a'	0.908 (41)
H6a2 ---C6a	0.922 (65)	H6a2'---C6a'	0.992 (39)
H6a3 ---C6a	0.884 (51)	H6a3'---C6a'	0.937 (44)
H10 ---C10	0.997 (58)	H10' ---C10'	0.976 (37)
H13 ---C13	1.010 (57)	H13' ---C13'	0.950 (38)
H14 ---C14	0.964 (48)	H14' ---C14'	0.945 (46)
H161---N16	1.000 (58)	H161'---N16'	0.814 (47)
H162---N16	0.945 (61)	H162'---N16'	0.836 (40)

ring are 17.2° and 21.4° for molecules **1** and **1'** respectively. The torsion angles along bonds of the N-N-CO-C(9) system are N(1)-N(7)-C(8)-O(8a) 0° and −0.9°, and N(1)-N(7)-C(8)-C(9) 178.6° and −178.9° for molecules **1** and **1'** respectively (Figure 2).

The differences between special torsion angles in molecules **1** and **1'** suggest that the rotation around C(11)-S(15), S(15)-N(16) and C(8)-C(9) bonds are relatively soft parameters which may be easily influenced by the crystal or by packing forces. Thus, the torsion angles are C(10)-

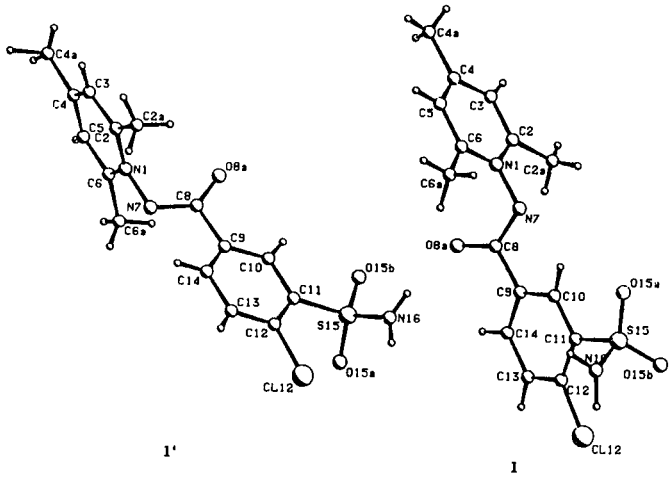


Figure 2. Views of the two crystallographically independent molecular conformations in the crystal structure of Besulpamide.

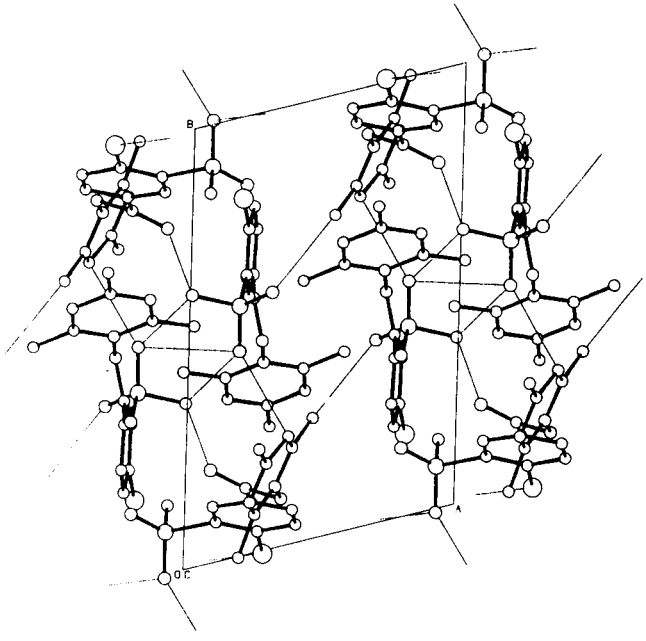


Figure 3. Unit cell and molecular packing of Besulpamide.

C(11)-S(15)-N(16) 112.6° and 126.1°, C(12)-C(11)-S(15)-N(16) −66.3° and −57.1°, C(11)-S(15)-N(16)-H(161) 79.8° and 86.1°, C(11)-S(15)-N(16)-H(162) −69.7° and −96.2°, N(7)-C(8)-C(9)-C(10) 17.8° and 158.2°, and N(7)-C(8)-C(9)-C(14) −163.3° and −20.4° for molecules **1** and **1'** respectively.

The bond lengths and angles in the trimethylpyridinium ring are in good agreement with other reported [20] trimethylpyridinium salts. The distances N-N are also in agreement with the values found in 1-nitroimidepyridines [21] (1.422 Å) or in N-N linked heterocycles [22] (1.414 Å).

The hydrogens available to form hydrogen bonds (SO_2NH_2) were found to be within an appropriate distance and in an appropriate direction to form them with suitable electronegative acceptors. In the crystal structure of the present betaine, one oxygen of the sulphonamide group of one molecule and the oxygen of the carbonyl of another molecule are proton acceptors from the hydrogens of the SO_2NH_2 group from a third molecule. Thus, the bond lengths N(16)-H(161)....O(8a') are 1.000 Å (N-H) and 1.843 Å (H....O).

EXPERIMENTAL

NMR Spectroscopy.

The nmr spectra were determined out in the pulsed Fourier transform mode with an internal deuterium lock, at 100 MHz (^{1}H) and 25.1 MHz (^{13}C) on a Bruker instrument, model AM-100. The spectra were recorded in deuteriochloroform or in DMSO-d_6 at concentrations 1% w/v (^{1}H) or 15-20% w/v (^{13}C). Operating temperature was 310°K. Heteronuclear decoupling experiments for correlation of protons with directly attached carbons (SFORD method) were carried out by collecting ^{13}C nmr FIDs under CW decoupling with a decoupler power setting of 40 H, *i.e.* a decoupler output attenuation of 40 dB below a nominal 11 w full power. The measurement of longrange selective heteronuclear ^{13}C{^{1}H} nOe enhancements was achieved by means of the microprogram previously described [13]. The selective generation of heteronuclear ^{13}C{^{1}H} nOe was carried out under CW decoupling with a decoupler power setting of 61L, *i.e.*, a decoupler output attenuation of 61 dB below a nominal 0.2 w full power. The irradiation time was usually 30-40 s except when OH were irradiated at compound **2** and **5** when it was 180 s. NOe enhancements factors were calculated from peak height ratios. The reference chosen was the central peak of DMSO-d_6. Thus $\eta_\delta = [(I/Iref)_N/(I/Iref)_B]-1$, where the subscripts N and B refer to the nOe spectrum and base spectrum, respectively.

Mass spectrometry.

Mass spectra were determined with a Hewlett Packard 5895 spectrometer using the direct insertion method and electron-impact at an ionizing voltage of 70 eV.

Crystal Data.

$C_{15}H_{16}ClN_3O_3S$, M = 353.82. Triclinic, a = 8.485(3), b = 14.282(2), c = 15.312(6) Å, α = 69.41(2), β = 82.22(4), γ = 72.78(3), V = 1 658.0(8) Å^3 (by least-squares refinement on diffractometer angles for 25 high angle reflections), λ = 0.71069 (Å), space group $P\bar{1}$, Z = 4, D_x = 1.417 g cm^{-3}. Pale yellow single crystals were grown from ethanol by slow evaporation of the solvent. Crystal dimensions: 0.20 x 0.30 x 0.35 mm, $\mu(M_o\text{-}K_\alpha)$ = 3.65 cm^{-1}.

Data Collection and Processing.

Enraf-Nonius CAD4 diffractometer, ω-2 θ mode, graphite-monochromated $M_o\text{-}K_\alpha$ radiation; 5 790 reflections measured ($-10 > h > 10$, $-17 > k > 17$, $0 > l > 18$), 4106 reflections observed with $I > 2.0\sigma(I)$. No absorption corrections were made. The intensity of the standard reflexion ($\bar{1}\ \bar{4}\ \bar{7}$) dropped by an average of 0.8% over the period of data collection.

Table 7

Bond Distances (Å) and Bond Angles (Degrees) with their e.s.d.'s

Molecule 1		Molecule 1'	
Bond	Length	Bond	Length
C2 ---N1	1.357 (5)	C2' ---N1'	1.357 (4)
C6 ---N1	1.362 (6)	C6' ---N1'	1.358 (3)
N7 ---N1	1.416 (5)	N7' ---N1'	1.415 (4)
C2a ---C2	1.493 (8)	C2a' ---C2'	1.490 (5)
C3 ---C2	1.383 (7)	C3' ---C2'	1.382 (5)
C4 ---C3	1.372 (8)	C4' ---C3'	1.377 (4)
C4a ---C4	1.505 (9)	C4a' ---C4'	1.515 (7)
C5 ---C4	1.376 (6)	C5' ---C4'	1.373 (6)
C6 ---C5	1.377 9 7)	C6' ---C5'	1.380 (5)
C6a ---C6	1.492 (6)	C6a' ---C6'	1.496 (6)
C8 ---N7	1.317 (5)	C8' ---N7'	1.319 (4)
O8a ---C8	1.261 (4)	O8a' ---C8'	1.248 (3)
C9 ---C8	1.505 (7)	C9' ---C8'	1.513 (4)
C10 ---C9	1.390 (5)	C10' ---C9'	1.388 (5)
C14 ---C9	1.378 (6)	C14' ---C9'	1.389 (4)
C11 ---C10	1.394 (7)	C11' ---C10'	1.395 (4)
C12 ---C11	1.392 (5)	C12' ---C11'	1.391 (4)
S15 ---C11	1.784 (3)	S15' ---C11'	1.784 (3)
CL12 ---C12	1.732 (5)	CL12' ---C12'	1.738 (3)
C13 ---C12	1.386 (5)	C13 ---C12'	1.390 (5)
C14 ---C13	1.388 (7)	C14' ---C13'	1.384 (5)
O15a ---S15	1.431 (3)	O15a' ---S15'	1.434 (2)
O15b ---S15	1.426 (3)	O15b' ---S15'	1.426 (2)
N16 ---S15	1.593 (5)	N16' ---S15'	1.592 (3)

Bonds	Bond Angle	Bond	Bond Angle
C6 -N1 -C2	122.2 (4)	C6' -N1' -C2'	122.2 (3)
N7 -N1 -C2	118.2 (4)	N7' -N1' -C2'	118.5 (2)
N7 -N1 -C6	119.5 (3)	N7' -N1' -C6'	119.2 (3)
C2a -C2 -N1	119.0 (4)	C2a' -C2' -N1'	118.6 (3)
C3 -C2 -N1	118.2 (4)	C3' -C2' -N1'	118.3 (3)
C3 -C2 -C2a	122.8 (4)	C3' -C2' -C2a'	123.1 (3)
C4 -C3 -C2	121.8 (4)	C4' -C3' -C2'	121.5 (4)
C4a -C4 -C3	120.7 (4)	C4a' -C4' -C3'	120.9 (4)
C5 -C4 -C3	117.5 (5)	C5' -C4' -C3'	117.9 (4)
C5 -C4 -C4a	121.8 (5)	C5' -C4' -C4a'	121.1 (3)
C6 -C5 -C4	121.9 (5)	C6' -C5' -C4'	121.4 (3)
C5 -C6 -N1	118.2 (4)	C5' -C6' -N1'	118.6 (3)
C6a -C6 -N1	117.9 (5)	C6a' -C6' -N1'	118.0 (3)
C6a -C6 -C5	123.9 (5)	C6a' -C6' -C5'	123.4 (3)
C8 -N7 -N1	112.2 (3)	C8' -N7' -N1'	111.2 (2)
O8a -C8 -N7	126.7 (4)	O8a' -C8' -N7'	128.9 (3)
C9 -C8 -N7	113.2 (3)	C9' -C8' -N7'	112.3 (2)
C9 -C8 -O8a	120.0 (3)	C9' -C8' -O8a'	118.8 (3)
C10 -C9 -C8	121.4 (4)	C10' -C9' -C8'	118.5 (2)
C14 -C9 -C8	120.2 (3)	C14' -C9' -C8'	121.8 (3)
C14 -C9 -C10	118.4 (4)	C14' -C9' -C10'	119.6 (3)
C11 -C10 -C9	121.6 (4)	C11' -C10' -C9'	120.3 (3)
C12 -C11 -C10	118.8 (3)	C12' -C11' -C10'	119.2 (3)
S15 -C11 -C10	118.6 (3)	S15' -C11' -C10'	118.0 (2)
S15 -C11 -C12	122.5 (3)	S15' -C11' -C12'	122.7 (2)
CL12 -C12 -C11	122.1 (3)	CL12' -C12' -C11'	120.9 (3)
C13 -C12 -C11	120.1 (4)	C13' -C12' -C11'	120.8 (3)
C13 -C12 -CL12	117.8 (3)	C13' -C12' -CL12'	118.2 (2)
C14 -C13 -C12	119.9 (4)	C14' -C13' -C12'	119.3 (3)
C13 -C14 -C9	121.2 (4)	C13' -C14' -C9'	120.8 (3)
O15a -S15 -C11	105.3 (2)	O15a' -S15' -C11'	106.5 (1)
O15b -S15 -C11	108.5 (2)	O15b' -S15' -C11'	106.4 (2)
O15b -S15 -O15a	119.0 (2)	O15b' -S15' -O15a'	119.0 (1)
N16 -S15 -C11	107.8 (2)	N16' -S15' -C11'	107.9 (2)
N16 -S15 -O15a	107.2 (2)	N16' -S15' -O15a'	108.2 (2)
N16 -S15 -O15b	108.5 (2)	N16' -S15' -O15b'	108.5 (1)

Structure Analysis and Refinement.

The structure was resolved by direct methods applying the MULTAN 11/84 [23] system, the E-map based on the phase set with ABS FOM = 1.196, PSI O = 1.955, RESID = 6.75 and CFOM = 2.77 established positions of 40 non hydrogen atoms for two independent molecules; a subsequent difference Fourier synthesis gave the positions for the remaining non H-atoms. The refinement of the structural model was performed by full-matrix

least-squares methods (SHELX-76) [24]. All hydrogen atoms were located from a difference Fourier synthesis and refined with global temperature factors. In the final difference Fourier map calculated after the last cycle the maximum and minimum heights were 0.22 and -0.28 e $Å^{-3}$. The weighting scheme $w = 1/[\sigma^2(F_o) + 0.000872\ F_o^2]$, with σ (F°) from counting statistics gave satisfactory agreement analyses. Final R and R_w values are 0.038 and 0.041. Scale factor 1.420 (2). Atomic scattering factors and corrections for anomalous dispersion were taken from the International Tables from X-Ray Crystallography [25]. Geometrical calculations were performed with XANADU [26] and the perspective stereoscopic view with PLUTO [27].

Compounds.

Compounds **1** and **3** were obtained as reported in reference 1.

1-[4-Chloro-2-hydroxy-5-sulfamoylbenzoyl)-amino]-2,4,6-trimethylpyridinium Hydroxide Inner Salt, **2**, and 1-Amino-2,4,6-trimethylpyridinium 2-carbonylhydrazide-5-chloro-4-sulfamoylphenolate, **5**.

A solution of freshly prepared trimethylpyrilium tetrafluoroborate (4.2 g, 0.02 mole) and 4-chloro-2-hydroxy-5-sulfamoylbenzoylhydrazide (5.3 g, 0.02 mole) in ethanol (100 ml) was refuxed for 6 hours and then stirred at 25° for 14 additional hours. To the stirred solution 85% potassium hydroxide (1.38 g, 0.021 mole) was added and then stirred for one hour at room temperature. The precipitate formed was filtered and washed with methanol at 40°. The filtrate was evaporated *in vacuo* and the solid was recrystallized from methanol to give white crystals of **5** (1.1 g, 14%), mp 219-221°.

Anal. Calcd. for $C_{15}H_{20}N_5ClSO_4$: C, 44.83; H, 5.02; N, 17.43; Cl, 8.82; S, 7.98. Found: C, 44.67; H, 5.28; N, 17.49; Cl, 8.82; S, 7.96.

The last filtrate was evaporated *in vacuo*. Column chromatography on silica gel eluting with chloroform/methanol (95:5) afforded a solid which was recrystallized from methanol to give white crystals of **2** (2.1 g, 28%), mp 260-261°.

Anal. Calcd. for $C_{15}H_{16}N_3ClSO_4$: C, 48.72; H, 4.36; N, 11.36; Cl, 9.59; S, 8.67. Found: C, 48.90; H, 4.63; N, 11.57; Cl, 9.51; S, 8.56.

Reaction of 1-[(4-Chloro-2-hydroxy-5-sulfamoylbenzoyl)amino]-2,4,6-trimethylpyridinium Hydroxide Inner Salt, **2**, with 4-Chloro-2-hydroxy-5-sulfamoylbenzoylhydrazide, **6**.

To a solution of **2** (370 mg, 1 mmole) and 4-chloro-2-hydroxy-5-sulfamoylbenzoylhydrazide, **6**, (266 mg, 1 mmole) in methanol (30 ml) and water (1 ml), a solution of potassium hydroxide in methanol was added (11 ml potassium hydroxide 0.2*M*, 2.2 mmoles). The stirred solution was refluxed for 24 hours and then concentrated to 30 ml. From this solution **5** was obtained by crystallization at room temperature and identified by ^{1}H-nmr. The filtrate was neutralized with hydrochloric acid and the precipitate removed by filtration. The last filtrate was acidified to *p*H = 3 with hydrochloric acid and a minute amount of a solid compound was removed by filtration. This compound was identified as 4-chloro-2-hydroxy-5-sulfamoylbenzoic acid, **7**, by ^{1}H-nmr: 7.15 (s, H-3), 7.50 (b, NH_2), 8.39 (s, H-6), 12.50 (b, COOH, OH).

Biological Activities.

The diuretic activity of **2** (dose 10 mg/kg, urinary volume 41.2 ml/kg, Na^+ 7.39 meqv/kg, K^+ 2.1 meqv/kg, Cl^- 9.25 meqv/kg, osmotic pressure 23.6 mosm/kg, *p*H 5.58) is very similar to that of Besulpamide [28], **1**. In contrast, the salt **5** lacks diuretic activity.

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REFERENCES AND NOTES

[1a] J. Esteve Soler, European Patent Application EP 117,196; *Chem. Abstr.*, **102**, 24490k (1985); [b] J. Esteve Soler, Spanish Patent ES 529,069; [c] J. Esteve Soler, French Demande FR 2,540,870; [d] J. Esteve Soler, U. S. Patent US 4,563,467.

[2] *Drugs of the Future*, **12**, 205 (1987).

[3] J. Esteve, L. Martínez, R. Roser and R. Sagarra, *Meth. Find. Exptl. Clin. Pharmacol.*, **9**, 121 (1987).

[4a] J. B. Bapat, R. J. Blade, A. J. Boulton, J. Epsztajn, A. R. Katritzky, J. Lewis, P. Molina-Buendia, P. L. Nie and C. A. Ramsden, *Tetrahedron Letters*, 2691 (1976); [b] R. Neidlein and P. Witerzens, *Arch. Pharm. (Weinheim)*, **309**, 649 (1976).

[5a] A. R. Katritzky and C. M. Marson, *Angew. Chem., Int. Ed. Engl.*, **23**, 420 (1984); [b] A. R. Katritzky, *Tetrahedron*, **36**, 679 (1980).

[6a] H. J. Timpe, *Adv. Heterocyclic Chem.*, **17**, 218 (1974); [b] Y. Tamura and M. Ikeda, *ibid.*, **29**, 71 (1981).

[7a] A. R. Katritzky and M. Kinns, *Org. Magn. Reson.*, **7**, 569 (1975); [b] R. M. Claramunt and J. Elguero, *Collect. Czech. Chem. Commun.*, **46**, 584 (1981); [c] A. T. Balaban, A. Dinculescu, F. Iordache, F. Chiraleua and D. Patraçcoiu, *Chem. Scr.*, **18**, 230 (1981).

[8a] A. Dinculescu, M. Fahmy, F. Chiraleu and A. T. Balaban, *Rev. Roum. Chim.*, **28**, 699 (1983); [b] R. A. Abramovitch and T. Takaya, *J. Org. Chem.*, **37**, 2022 (1972).

[9] Figures in brackets are $\Delta\ \delta$, *i.e.* δ **2**- δ **1** for the same proton.

[10] E. Pretsch, T. Clerc, J. Seibl and W. Simon, "Tablas para la elucidación estructural de compuestos orgánicos por métodos espectroscópicos", Ed. Alhambra, Barcelona, 1980, pp H255.

[11] R. J. Highet and P. F. Highet, *J. Org. Chem.*, **30**, 902 (1965).

[12] S. A. Dias, A. W. Downs and W. R. McWhinnie, *J. Chem. Soc., Dalton Trans.*, 162 (1975).

[13a] E. Breitmeier and W. Voelter, "Carbon-13 NMR Spectroscopy", 3rd Ed, Verlag Chemie, Weinheim, 47 (1987); [b] *ibid.*, 75; [c] *ibid.*, 117.

[14a] F. Sánchez-Ferrando, *Magn. Reson. Chem.*, **23**, 185 (1985); [b] C. Cativiela and F. Sánchez-Ferrando, *Magn. Reson. Chem.*, **23**, 1072 (1985).

[15a] J. Frigola, A. Colombo, J. Más and J. Parés, *J. Heterocyclic Chem.*, **24**, 399 (1987); [b] J. Frigola, *J. Chem. Soc., Perkin Trans. 2*, 241 (1988).

[16] J. Cervello, M. Gil, P. de March, J. Marquet, M. Moreno-Mañas, J. L. Roca and F. Sánchez-Ferrando, *Tetrahedron*, **43**, 2381 (1987).

[17] Figures in brackets are $\Delta\ \delta$, *i.e.*, δ **2**- δ **1**, for the same carbon.

[18] M. Ikeda, N. Tsujimoto and Y. Tamura, *Org. Mass. Spectrom.*, **5**, 61 (1971).

[19] M. J. O'Hare, E. Tan, C. Rehm, P. Grebow, B. Loev and E. Neiss, *J. Heterocyclic Chem.*, **20**, 819 (1983).

[20] A. Camerman, L. H. Jensen and A. T. Balaban, *Acta Cryst.*, **B25**, 2623 (1969).

[21] J. Arriau, J. Deschamps, J. R. C. Duke, J. Epsztajn, A. R. Katritzky, E. Lunt, J. W. Mitchell, S. Q. A. Rizvi and G. Roch, *Tetrahedron Letters*, 3865 (1974).

[22] M. P. Sammes, T. F. Lai, A. R. Katritzky, R. Murugan and H. Luce, *J. Chem. Soc., Perkin Trans II*, 573 (1985) and references therein.

[23] P. Main, G. Germain and M. M. Woolfons, "MULTAN 11/84. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data", University of York, England, 1984.

[24] G. M. Sheldrick, "SHELX 76. Program for Crystal Structure Determination", University of Cambridge, England, 1976.

[25] "International Tables for X-Ray Crystallography", Kynoch Press, Birmingham, 1974.

[26] P. Roberts and G. M. Sheldrick, "XANADU. Program for Crystallographic Calculations", University of Cambridge, England, 1975.

[27] W. D. S. Motherwell and W. Clegg, "PLUTO. Program for Plotting Molecular and Crystal Structures", University of Cambridge, England, 1978.

[28] M. Colombo, A. J. Farré, M. Fort, L. Martínez, R. Roser and R. Sagarra, *Meth. Find. Exptl. Clin. Pharmacol.*, **9**, 101 (1987).