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METHANOLYSIS PRODUCTS OF SULFOMYCIN I [1)]

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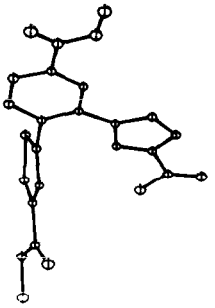
In the previous report[2)], we described hydrolysis products of sulfur-containing antibiotic sulfomycin I. In the present report, sulfomycinamic acid and sulfomycinic acid obtained by methanolysis of sulfomycin I are to be dealt with.

Sulfomycin I was refluxed with the cation exchange resin Amberlyst-15 for 20 hours in absolute methanol. The filtrate of reaction mixture was evaporated to dryness and the residue was separated by silica gel column chromatography (solvent system: 2% methanol-chloroform). Thus, crude crystals of three major components dimethyl sulfomycinamate (1), methyl sulfomycinate (2) and sulfomycinic amide (3) were obtained. Threonine methyl ester was further eluted with methanolic hydrogen chloride from the resin.

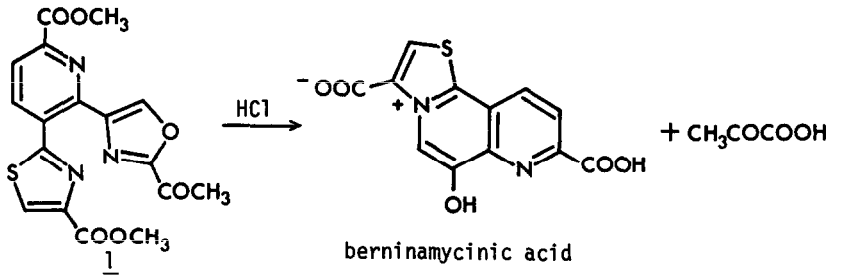
Crystals of 1 were recrystallized from chloroform-ethanol as colorless needles: mp 160.5-161.0°; $C_{17}H_{13}N_3O_6S$; uv (MeOH) 255 (log ε 4.32), 285 nm (sh.); ir (KBr) 1720, 1700 cm^{-1}; nmr ($CDCl_3$) 2.46 (3H, s, CH_3CO-), 3.96 and 4.03 (3H, s, CH_3OCO-), 8.19 (2H, s), 8.34 (1H, s), 8.37 ppm (1H, s); ms *m/e* 388 ($M^+ + 1$), 356 ($M^+ - OCH_3$), 344 ($M^+ - COCH_3$, base peak), 328 ($M^+ - COOCH_3$), 285. These data suggested that 1 was a heteroaromatic compound possessing two methoxycarbonyl and one acetyl groups. Acid hydrolysis of 1 (6 hours at 110° in 6N aqueous hydrochloric acid) afforded berninamycinic acid and pyruvic acid, which were, as reported previously, obtained by acid hydrolysis of sulfomycin I. The structure of 1 was elucidated by X-ray crystallographic analysis and the resulting molecular structure shown in Figure 1 was drawn by program "ORTEP"[3)].

A crystal of dimentions 0.3 x 0.2 x 0.5 mm was selected for the X-ray examination. This crystal was monoclinic, ($a = 13.233$, $b = 20.074$, $c = 6.620$ Å, $\beta = 99.16°$ and $Dc = 1.482$ g/cm^3) and the space group was $P2_1/a$ with four molecules per unit cell. A total of 3047 reflections of which 2091 intensities were greater than 3 sigma were measured with automatic goniometer AFC/3 (RIGAKU) using Cu Kα radiation with a graphite monochromator. The structure was solved by direct methods with MULTAN [4], and block-diagonal least-squares refinement of the non-hydrogen atoms with anisotropic temperature factor for all atoms gave the final R-value of 0.139 for all observed reflection.

Figure 1



Scheme 1



1 berninamycinic acid

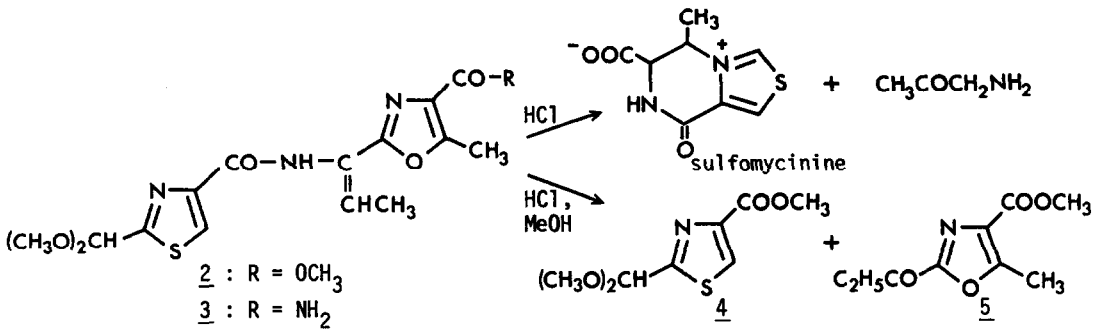
The novel structure assigned to sulfomycinamic acid correlates well with the spectrometric result and explains rationally the formation of its hydrolysis products [5].

The physicochemical properties of 2 were as follows : colorless needles; mp 124.0-124.5°; $C_{16}H_{19}N_3O_6S$ (*m/e* 381.0979, Δ = -1.3 mmu); uv (MeOH) 247 nm (log ε 4.38); ir (KBr) 1715, 1685 cm^{-1}; nmr ($CDCl_3$) 1.87 (3H, d, J = 7.2 Hz, C<u>H</u>$_3$CH=), 2.61 (3H, s, C<u>H</u>$_3$-Ar), 3.46 (6H, s, (C<u>H</u>$_3$O)$_2$CH-), 3.86 (3H, s, C<u>H</u>$_3$OCO-), 5.58 (1H, s, -C<u>H</u>(OCH$_3$)$_2$), 6.68 (1H, q, J = 7.2 Hz, =C<u>H</u>CH$_3$), 8.20 (1H, s), 8.70 ppm (1H, br s, -N<u>H</u>-).

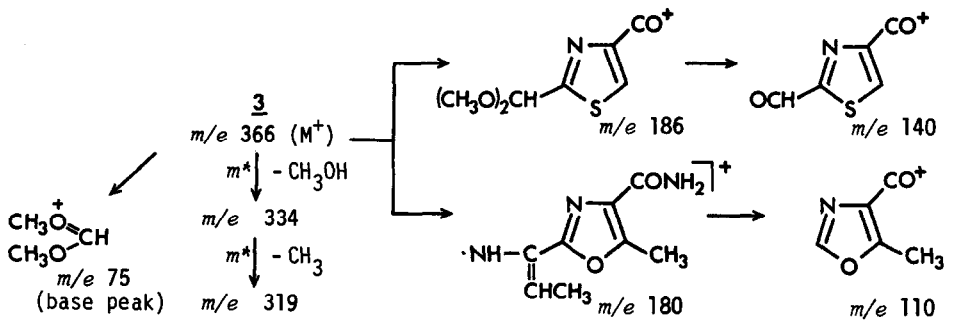
Another methanolysis product 3 : $C_{15}H_{18}N_4O_5S$ (*m/e* 366.1014, Δ = 1.7 mmu); mp 194.5-195.0°, showed properties similar to 2. By acid hydrolysis, both compounds gave sulfomycinine and aminoacetone. The nmr spectrum of 3 was similar to that of 2, but the methoxycarbonyl group signal at 3.86 ppm was not observed and, instead, new amino proton signals appeared at 6.05 and 6.95 ppm. These data suggested that 3 was the compound with the amide group in place of the methyl ester of 2 and this assumption was verified by converting 2 to 3 by ammonolysis.

Presumably, 3 was the initial degradation product but was converted to 2 upon prolonged reaction. The structure of 3 was assigned as follows.

Scheme 2



Scheme 3



By a usual method, 3 was turned to 2,4-dinitrophenylhydrazone : $C_{19}H_{16}N_8O_7S$ (*m/e* 500.0846, Δ = -1.4 mmu) [$(CH_3O)_2CH$- → 2,4-DNP=CH-]. The mass spectra of 3 and 2,4-DNP of 3 suggested the presence of thiazole ring substituted with a dimethoxymethyl group. In reduction with Mg-MeOH, 3 was converted to the dihydro compound : $C_{15}H_{20}N_4O_5S$ (M^+ 368), which gave α-aminobutyric acid by acid hydrolysis. Based upon these data and also judging from the isolation of sulfomycinine by acid hydrolysis of 3, the presence of 1-(4-thiazolylcarboxamido)-1-propenyl unit in 3 was reasonable. Furthermore, the nmr spectrum of 3 showed signals corresponding to an aromatic methyl and a carboxamide groups. The above propenyl unit together with methyl and carboxamide groups accounted for $C_{12}H_{18}N_3O_4S$. Therefore, only C_3NO was left to complete the molecular formula of 3. The C_3NO unit would be an oxazole or an isoxazole which was, as indicated by the mass fragmentation of 3 (Scheme 3), substituted with methyl, carboxamide and aminopropenyl

groups. Isolation of aminoacetone by hydrolysis excluded the isoxazole structure and made possible to determine the positions of substituents on the oxazole ring. The position of dimethoxymethyl group was suggested to be at C-2 of thiazole, judging from the chemical shift of the aromatic proton (8.20 ppm), which was assignable to the C-5 proton of thiazole rather than to the C-2 proton. Based on the arguments presented above, the structure of 2 and 3 could be assigned as depicted (Scheme 2) [5].

To confirm the structure, 3 was further methanolyzed with methanolic hydrogen chloride. The compound 3 was cleaved into two components, 4 and 5, which were assigned as 4-carbomethoxy-2-dimethoxymethylthiazole and 4-carbomethoxy-5-methyl-2-propionyloxazole [6], respectively, on the basis of their spectral data; 4 : $C_8H_{11}NO_4S$; liquid; ms *m/e* 216 ($M^+ - 1$), 140, 75; uv (MeOH) 236nm; nmr ($CDCl_3$) 3.48 (6H, s), 4.00 (3H, s), 5.64 (1H, s), 8.22 ppm (1H, s), 5 : $C_9H_{11}NO_4$; mp 71.0-71.5°; ms *m/e* 197 (M^+), 110; uv (MeOH) 227 (log ε 3.93), 265 nm (4.04); nmr ($CDCl_3$) 1.24 (3H, t, J = 8 Hz), 2.76 (3H, s), 3.18 (2H, q, J = 8 Hz), 4.01 ppm (3H, s).

Recently, berninamycin A was reported to be composed of the berninamycyl unit, retaining the zwitterion as such [7]. Isolation of sulfomycinamic acid, however, indicated that the thiazole and pyridine rings in sulfomycin I were present as the sulfomycinamyl unit and not as the berninamycyl unit, in analogy with the structure of the related fragments of the thiostrepton family antibiotics, *e.g.* nosiheptide [8].

REFERENCES and NOTES

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5) ^{13}C nmr study also supported the structure of 1 and 2.

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