can only be measured when extensive isomerization of the arenonium ions formed in the kinetically significant step of the attack is prevented by a suitable choice of the reaction conditions.

It cannot be claimed that the present data fully reflect the inherent positional selectivity of gaseous *i*-$C_3H_7^+$, since, even under conditions specifically chosen to minimize secondary isomerization, its occurrence cannot be entirely excluded.

However, we feel that even the lower selectivity limit established in the present study, together with the results of previous investigations, indicates that the lack of positional selectivity *is not* a distinctive feature of the gas-phase alkylation and that the *i*-$C_3H_7^+$ cation represents no exception when compared to other gaseous electrophiles.

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Structure of A204A, a New Polyether Antibiotic

Sir:

Antibiotic A204A is the major factor of a group of closely related, biologically active compounds produced by a strain of *Streptomyces albus*.[1] It is active against Gram-positive bacteria, fungi, and several plant pathogens, and it is effective in the treatment of coccidial infections in poultry. In addition, A204A induces monovalent cation permeability in rat liver mitochondria.[2] In light of its biological activity and its physical properties, which are outlined below, A204A was thought to belong to the family of polycyclic, polyether, monocarboxylic acid antibiotics, which includes monensin,[3] nigericin,[4] X-537A,[5] grisorixin,[6] dianemycin,[7] and X-206.[8]

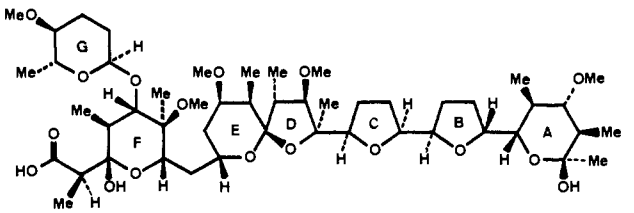
A204A is a monocarboxylic acid: mp 96–98°; $pK_a' = 6.1$ (66% DMF); $[\alpha]^{25}D$ +68.1° (*c* 2, MeOH); ν_{max} ($CHCl_3$) 1681 cm^{-1} (CO_2H); no uv maximum beyond 210 nm. The sodium salt of A204A (mp 144–145°; $[\alpha]^{25}D$ +55.0° (*c* 2, MeOH); ν_{max} ($CHCl_3$) 1600 cm^{-1} (CO_2^-)) and the free acid are insoluble in water but soluble in organic solvents. The nmr spectrum ($CDCl_3$) showed the presence of five methoxyl groups at δ 3.30–3.43, a number of *C*-methyl groups and protons attached to carbon atoms bearing oxygen, and a broad singlet at δ 4.85.

The silver and sodium salts of A204A crystallize from acetone–water as colorless, triangular plates which contain one molecule of acetone per molecule of antibiotic. The crystal data for the two nearly isomorphous salts are given in Table I. A total of 2409 unique reflections for the silver salt and 2457 for the sodium salt were measured using an automated diffractometer with filtered copper radiation. The structure of the silver salt was solved by the heavy atom technique, albeit with great difficulty because of the persistent pseudosymmetry. The structure of the silver salt has been refined to an *R* value of 0.15 by least-squares methods using anisotropic temperature factors for the heavy atom and isotropic factors for all other atoms. The structure of the sodium salt was refined using anisotopic temperature factors for the sodium and oxygen atoms to give an *R* value of 0.14. Further refinement has been hampered by the limited data available and by the apparent disorder of the acetone solvate molecule. The atomic parameters for the two salts are given in Table II, with the atoms numbered as in Figure 1.[9] The absolute configuration of the molecule was determined for the silver salt by anomalous dispersion.

Table I. Crystal Data for Silver and Sodium Salts of A204A, $C_{49}H_{83}O_{17}\cdot M\cdot C_3H_6O$

	M	
	Ag	Na
a	26.971 (4) Å	27.539 (4) Å
b	14.517 (2) Å	14.515 (2) Å
c	14.419 (2) Å	14.406 (2) Å
β	91.94 (1)°	92.36 (1°)
Space group	*C*2	*C*2
Molecules/cell	4	4
Obsd density	1.293 g cm^{-3}	1.190 g cm^{-3}
Obsd mol wt	1099	1031
Calcd mol wt	1110	1025

The free acid form (**1**) of A204A has the empirical



1

formula $C_{49}H_{84}O_{17}$ (mol wt 945.2), making it the largest of the known, naturally occurring, polyether antibiotics. The compound is a monocarboxylic acid, and, like nigericin, grisorixin, and dianemycin, it has a 30-carbon backbone. Rings A–F are very similar in structure and stereochemistry to nigericin and grisorixin, and ring G occurs identically in dianemycin.

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(9) A list of the atomic parameters (Table II) will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-73-3399.

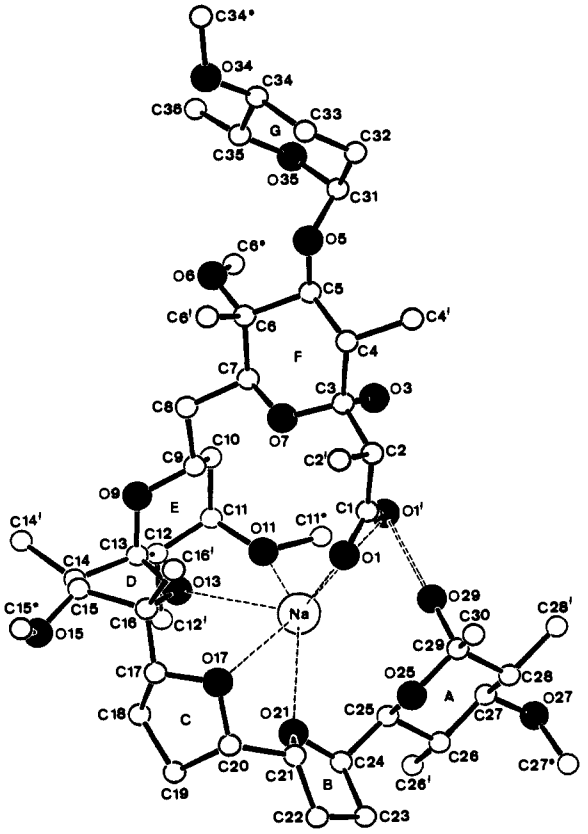

Figure 1.

The structure of the sodium salt of A204A is shown in Figure 1. The acetone solvate molecule, which is not shown, is weakly hydrogen bonded to the hydroxyl on ring F. The molecule has a central cavity of 5–6 Å in diameter formed by fastening the ends of the chain together with a hydrogen bond (double dotted line in Figure 1) between the hydroxyl on ring A and one of the carboxylate oxygen atoms. Six oxygen atoms of the antibiotic molecule lie less than 3.0 Å from the sodium ion and may be considered the principal ligands (single dotted lines). The structure of the complex is very similar to that found for grisorixin, the additional ring G in A204A being well removed from the region of the central cavity.

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Reaction of Indoles with a Diazonium Salt (Fast Red B)

Sir:

Recent affinity labeling studies on rabbit antisaccharide antibodies[1] and staphylococcal nuclease[2] with diazotized aromatic amines have suggested—primarily on the evidence of visible absorption spectra—that a tryptophan residue has undergone reaction. This departure from the usual reaction of diazonium salts with protein-bound tyrosine, histidine, or lysine residues was first encountered in the alkaline coupling of proteins with diazotized arsanilic[3,4] or sulfanilic[5,6] acid. Reactions with indole,[5] indole-3-acetic acid,[4] and tryptophan[3,6] were also reported. Bernard[7] and Lillie[8] have discussed histochemical reactions of diazotized sulfanilic acid and *p*-nitroaniline, respectively, *vis-à-vis* tryptophan in proteins, reporting reactions with *N*-acetyltryptophan,[7] gramicidin,[7] and tryptamine.[8]

Fischer, in the first published study on the coupling of diazonium salts with indoles, observed[9] that 2-methylindole reacted rapidly to yield a crystalline 1:1 azo compound, whereas skatole, by presumably an alternate pathway, reacted more slowly to afford an uncharacterized product. This observation and Fischer's conclusion that reaction occurs preferentially at the indole 3 position were confirmed later[10] by Pauly and Gundermann with diazotized sulfanilic acid on indole, tryptophan, and 2-methyl-, 3-methyl- and 2,3-dimethylindole, and has been accepted in subsequent studies on the reaction of indole[11–17] and 2-methyl-[11,13–15] and 2-phenyl-[11] indole with various diazonium salts, though no additional experimental verification has been offered. A monosubstituted crystalline product from skatole and diazotized arsanilic acid has been described, solely by analogy to the pyrroles, as a coupling product at the 2 position.[15] In nearly all of these studies, the coupling reactions were conducted under weakly alkaline conditions, although there appears to be no justification for this practice.[18]

Because of our interest in the affinity labeling of proteins and certain histochemical applications we have examined the *acidic* and *neutral* reactions of skatole and 1,2- and 2,3-dimethylindole with a commercially available[19] diazonium salt (**1**) derived from 2-methoxy-4-nitroaniline.

The reaction with skatole was found to be pH dependent. When 2-deuterioskatole[20] was treated with 25%

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(18) Since simple indoles are un-ionized in the pH range under discussion, only the concentration of the reactive diazonium ion (RN_2^+) in equilibrium with unreactive $RN{=}NO^-$ is pH sensitive. As the pH increases the concentration of RN_2^+ decreases; *cf.* H. Zollinger, "Azo and Diazo Chemistry—Aliphatic and Aromatic Compounds," Interscience, New York, N. Y., 1961, p 47. Lillie has reported[8] a diazo coupling reaction with tryptamine at pH 4.
(19) Fast Red Salt B, Verona Dyestuffs, Union, N. J. 07083. Standardization either by comparison of the OD_{372} of a weighed amount in ethanol *vs.* a freshly prepared solution of diazotized (HCl) *p*-nitro-*o*-anisidine *or* coupling of a weighed amount with an excess of aniline and measurement of the OD_{530} in alkaline 50% aqueous dioxane gives 18–19% *p*-nitro-*o*-anisidine equivalents by weight.
(20) T. F. Spande, A. Fontana, and B. Witkop, unpublished work.