COMMUNICATIONS TO THE EDITOR

ON THE MUCOPEPTIDE FRACTION OF STREPTOCOCCAL CELL WALLS

Sir:

When whole or trypsin-treated walls of Group A hemolytic streptococci are extracted with hot formamide,[1] the group-specific C-polysaccharide is solubilized. The insoluble residue consists of particles retaining the size and shape of the original cell walls as shown by electron micrography.[2] The composition of the formamide-insoluble cell wall residue[2] reflects quite closely the mole ratios reported for cell walls exposed to trypsin.[3] The principal amino acids of the cell wall residue were found to be lysine, glutamic acid, and alanine; in addition, glucosamine and muramic acid were present. The mole ratios of the amino acids, in the order listed, approached the values 1:1:3. In their recent paper,[2] McCarty and Krause also described enzymatic solubilization of cell wall residue preparations.

We have analyzed cell wall residue preparations resulting from hot formamide extraction of trypsin-treated walls of *Streptococcus pyogenes*, Group A, Type 14, Strain S 23 by means of hydrolysis and quantitative paper chromatography. The results appear in Table I; amino acids other than those listed were absent or present in trace amounts only. The values found gravitate toward a gross composition 1:1:4:1:1 (lys, glut, ala, glucosamine, muramic acid).

TABLE I
COMPOSITION OF THE INSOLUBLE RESIDUE FROM STREPTOCOCCAL CELL WALLS AFTER TRYPSIN TREATMENT AND EXTRACTION WITH FORMAMIDE

	Mole ratios[a]
Lysine	1.01 ± 0.16 (9)
Glutamic acid	1.00 (9)
Alanaine	4.03 ± 0.45 (9)
Glucosamine	1.05 ± 0.07 (5)
Muramic acid	0.99 ± 0.20 (8)

[a] Two different cell wall residue preparations were analyzed; the figures in parentheses indicate the total number of "spots" of the particular substance that were cut out and evaluated; the values given are averages, together with the average of the highest negative and positive deviations from the mean.

The cell wall residue is solubilized rapidly by hot 4 N hydrochloric acid, but appreciable quantities of small, ninhydrin-reactive fragments were not seen until heating had been continued for 2–3 hours, when a rather complex mixture resulted.

Enzymatic solubilization was attempted with Maxted's enzyme,[4] phage-associated lysin,[5] and lysozyme. Maximum dissolution of hexosamine-containing material (85% of the total hexosamine present) was achieved by the action of lysozyme on a cell wall residue preparation that had been treated with aqueous acetic anhydride and then with dilute alkali. This treatment was designed to N-acetylate all non-acylated hexosamine constituents, and to saponify any O-acyl groups present, since O-acylation is known to decrease susceptibility to lysozyme.[6]

It should be noted that C-polysaccharide preparations obtained as stated, exhibit an absorption band in the ester-carbonyl region of the infrared spectrum; this band disappears on gentle treatment with alkali. By means of the hydroxamate method, the acyl groups were identified as formyl groups. The cell wall residue (after formamide treatment) was shown to contain saponifiable formyl and, to a lesser extent, actyl groups, whereas the original cell wall preparation did not give rise to formhydroxamic or acethydroxamic acid, nor did an ester-carbonyl band appear in the infrared spectrum of the trypsin-treated cell walls. Evidently, the extraction procedure introduces saponifiable formyl groups; the acetyl esters may result from an acetyl migration.

The lysozyme lysate contained only small amounts of material that reacted with ninhydrin, the Morgan–Elson reagent, or silver hydroxide. The material was fractionated according to its solubility in 66% and 85% alcohol; the most soluble portion was richest (29%) in hexosamine, the least soluble one, poorest (10%). The most soluble fraction was chromatographed on Whatman #3-MM paper with 1-butanol–acetic acid–water 4:1:5. Most of the material remained immobile at the site of application, and only two ninhydrin-negative, reducing (Ag) zones were detected.

The more abundant one of these contained about 5% of the total hexosamine solubilized from the cell wall residue. The material appeared homogeneous on re-chromatography in 1-butanol–acetic acid–pyridine–water and on electrophoresis on paper in 0.05 M borate at pH 10, 800 v., for two hours; in the electrical field, it migrated slowly at about six-tenths the rate shown by N-acetylglucosamine. At pH 3.5 or 10.0 (2 N acetic acid and 0.1 M carbonate, respectively), the fragment did not migrate. The composition was determined prior to and after electrophoresis on glass fiber sheets, with the results appearing in Table II.

TABLE II
COMPOSITION OF LYSOZYME-RELEASED FRAGMENT

	Molar ratio[a]
Alanine	1.0 (6)
Glucosamine	0.94 ± 0.07 (6)
Muramic acid	0.77 ± 0.06 (6)

[a] See, *mutat. mutand.*, note [a] in Table I.

The lysozyme-released fragment was treated with beta-N-acetylglucosaminidase[7] from hog epididymis with the production of two reducing

(1) A. T. Fuller, *Brit. J. Exptl. Pathol.*, **19**, 130 (1938).

(2) M. McCarty and R. M. Krause, *J. Exp. Med.*, **114**, 127 (1961).

(3) J. A. Hayashi and S. S. Barkulis, *J. Bact.*, **77**, 177 (1959).

(4) W. R. Maxted, *Lancet*, **2**, 255 (1948). A commercial preparation from Consolidated Laboratories, Inc., Chicago, was used.

(5) R. M. Krause, *J. Exp. Med.*, **108**, 803 (1958).

(6) W. Brumfitt, *Brit. J. Exp. Pathol.*, **40**, 441 (1959).

(7) J. Findlay and G. A. Levvy, *Biochem. J.*, **77**, 170 (1960). We welcome this opportunity to thank Dr. Levvy for his generous gift of a sample of his enzyme preparation.

entities (Ag), separable by chromatography in Partridge mixture and by electrophoresis in borate, pH 10. One of the products migrated like N-acetylglucosamine and reacted likewise in the Morgan–Elson test. The other product was again subjected to hydrolysis and analysis and was found to contain only alanine and muramic acid.

When the chromatographically immobile portion of the lysozyme lysate was subjected to brief, mild treatment with acid, there appeared several small reducing fragments, among them glucosamine and N-acetylglucosamine, as well as an additional reducing and ninhydrin-negative entity. Hydrolysis of this material liberated glucosamine and alanine in the ratio of approximately 2:1.

We cannot, at this time, decide what the detailed structures of the fragments are, and which, if any, of the conceivable structures may occur in the native cell wall. However, we feel that the observations presented give new knowledge about the association, with one another, of streptococcal cell wall constituents.

* CIBA Fellow

RESEARCH DEPARTMENT   H. HEYMANN
CIBA PHARMACEUTICAL PRODUCTS, INC.   L. D. ZELEZNICK*
SUMMIT, NEW JERSEY   J. A. MANNIELLO
                   RECEIVED NOVEMBER 2, 1961

ARYL FLUOROALKYL ETHERS AND SULFIDES: EVIDENCE FOR SULFUR d-ORBITAL INTERACTION

Sir:

We wish to report a new general method for the synthesis of aryl perfluoroalkyl ethers, the results of quantitative measurements demonstrating that fluoroalkoxy groups may be considered as halogen-like and that sulfur d-orbital interaction is significant when sulfur is bonded to the strongly electron-withdrawing fluoroalkyl group.

Aryl trifluoromethyl ethers have been synthe·· sized by the reaction of hydrogen fluoride or antimony fluorides with aryl trichloromethyl ethers[1] which were prepared by chlorination of the anisoles or phenyl esters of chlorothiocarbonic acid. It now has been found that the reaction of sulfur tetrafluoride[2] (hydrogen fluoride catalyst) with aryl fluorocarbonates[3] and perfluoroalkyl esters[4] provides a general, direct synthesis of aryl perfluoroalkyl ethers.[5] For the preparation of aryl tri-

$$\overset{O}{\underset{\|}{ArOCX}} + SF_4 \xrightarrow{HF} ArOCF_2X \quad (\text{where } X = F \text{ or } R_f)$$

fluoromethyl ethers, a convenient procedure is to react the phenols with carbonyl fluoride[3,6] in a Hastelloy autoclave at 100°. Without isolation of the fluorocarbonate, the sulfur tetrafluoride

then is added and the reaction mixture heated for several hours at 150–175°. The hydrogen fluoride by-product from the carbonyl fluoride reaction serves as catalyst for the reaction. For phenol and m- and p-nitrophenol, the yield of the ether is 60 to 80% over-all for the two steps. The reaction is general for substituted phenols including hydroquinone and resorcinol, provided that the substituents or the aromatic ring do not react with hydrogen fluoride or sulfur tetrafluoride.

The ionization constants of the trifluoromethoxy- and trifluoromethylthio-anilinium ions and benzoic acids[7,8] have been determined by standard literature methods[9,10,11] and are reported in Table I. The calculated σ-parameters are given in Table II and compared with those of several other substituents. The inductive and resonance contributions of the groups can be evaluated from σ_I and σ_R-parameters calculated according to Taft and Lewis.[12] From these results it can be seen that the OCF_3 group is very much like Cl, in that it withdraws electrons inductively but supplies them by resonance. Over-all it is a slightly stronger deactivating group than the halogens.[13]

TABLE I

IONIZATION CONSTANTS

Substituent X	Ionization constants ($-\log h$) (25°) of $XC_6H_4CO_2H$ in 50% ethanol	$XC_6H_4NH_3^+$ in water
m-OCF₃	5.14	3.25
p-OCF₃	5.19	3.82
m-SCF₃	5.13	3.30
p-SCF₃	4.98	2.78

The SCF_3 group has a σ_m value similar to that of the OCF_3 group, but unexpectedly has a more positive σ_p value with an added positive increment of 0.13 for the σ_p from ionization of anilium ions over the benzoic acids. This result is indicative of a $+R$ group. Recently Beishline[14] reanalyzed the ionization constant data for the SCH_3, $SCOCH_3$ and SCN groups on the basis of Taft's σ_R param-

(7) The aryl trifluoromethyl sulfides have been prepared by fluoride replacement in aryl trichloromethyl sulfides [see L. M. Yagupolsky and M. S. Marenets, J. Gen. Chem. USSR (English Trans.), 22, 2273 (1952)]. The reaction of aryl Grignard reagents with trifluoromethyl-sulfenyl chloride is an improved route to this class of sulfide and will be described in a future publication (see W. A. Sheppard, Abstracts of American Chemical Society Meeting, Chicago, Illinois, September. 1961, p. 7-M.

(8) The aryl perfluoroalkyl ethers and sulfides have been shown to have stability comparable to benzotrifluoride and the perfluoroalkoxy and perfluoroalkylthio groups are inert to normal chemical transformations involving the aromatic residue. Thus, the anilines and benzoic acids were prepared by catalytic reduction of the nitro derivatives and oxidation of the tolyl compounds. Both the trifluoromethoxy and trifluoromethylthio⁷ groups have been shown to be o,p-directing to electrophilic aromatic substitution.

(9) A. Bryson, J. Am. Chem. Soc., 82, 4858 (1960).

(10) J. D. Roberts, R. L. Webb and E. A. McElhill, ibid., 72, 408 (1950).

(11) A preliminary communication of pKa measurements on the above benzoic acids was recently reported by L. M. Yagupolsky and L. M. Yagupolskaya, Proc. Acad. Sci. (English Trans. from Doklady Akad. Nauk SSSR), 134, 1207 (1960)) and values are essentially in agreement with those reported in Table I. The only discussion of these results was the comment that the SCF₃ and OCF₃ groups were like halogens.

(12) R. W. Taft, Jr., and I. C. Lewis, J. Am. Chem. Soc., 81, 5343 (1959).

(13) The designation "super-halogen" has been suggested to describe this behavior of the perfluoroalkoxy groups.

(14) R. R. Beishline, J. Org. Chem., 26, 2533 (1961).

(1) (a) British Patent 765,527 (1957). (b) L. M. Yagupolsky and V. I. Troitskaya, J. Gen. Chem., USSR (English Trans.), 27, 518 (1957). (c) N. N. Iarovenko and A. S. Vasileva, J. Gen. Chem., USSR (English Trans.), 28, 2539 (1958).

(2) (a) C. W. Tullock, F. S. Fawcett, W. C. Smith and D. D. Coffman, J. Am. Chem. Soc., 82, 539 (1960). (b) W. R. Hasek, W. C. Smith and V. A. Engelhardt, ibid., 82, 543 (1960).

(3) H. J. Emeleus and J. F. Wood, J. Chem. Soc., 2183 (1948).

(4) R. F. Clark and J. H. Simons, J. Am. Chem. Soc., 75, 6305 (1953); M. Green, Chem. and Ind., 435 (1961).

(5) All new compounds have been characterized by analysis and spectral properties.

(6) M. W. Farlow, E. H. Man and C. W. Tullock, Inorganic Syntheses, 6, 155 (1960).