TABLE I

STUDIES CONCERNED WITH THE DIRECT PREPARATION OF BENZYLLITHIUM

	Lithium, type	Lithium, g. atom	Benzyl chloride, mole	Solvent	Temp., °C.[a] during addition	Addition[b] time, hr.	Products[c]
1	Ribbon	1.18	0.0952	Dioxane	20	0.25[d]	Bibenzyl (57.2%) Phenylacetic acid, 0.048 g.
2	Ribbon	0.783	.047	Dioxane	10[e]	1[f]	Bibenzyl (69%) Phenylacetic acid, 0.089 g.
3	Ribbon	1.04	.047	Dioxane	10	1.5[g]	Bibenzyl (67.5%) Phenylacetic acid, 0.068 g.
4	Ribbon	0.663	.047	Dioxane	8	1[h]	Bibenzyl not isolated Phenylacetic acid, trace
5	Wire	.310	.063	Tetrahydrofuran	−25	0.75[i]	Bibenzyl (72.8%) No acidic material[j]
6	Ribbon	.797	.047	Tetrahydrofuran	− 5	1[k]	Bibenzyl (75.5%) No acidic material[j]
7	Wire	.563	.047	Ethylene glycol dimethyl ether	−25	1[m]	Bibenzyl (85%) No acidic material[j]

[a] Initially the reaction was started at room temperature and the remaining benzyl chloride solution was added at the temperature listed. [b] The time required to add the benzyl chloride. [c] No attempt was made to recover any unreacted benzyl chloride in any run. [d] Aliquots were carbonated every 15 minutes during the addition. [e] The first one-half of the benzyl chloride solution was added between 20 and 25° while the remaining one-half was added at 10°. [f] The reaction mixture was carbonated immediately when the addition of benzyl chloride was completed. [g] The reaction mixture was stirred for 15 minutes after completing the addition of benzyl chloride. [h] The mixture was stirred at 0° for 1.5 hours prior to carbonation. [i] The reaction mixture was stirred at −50° for 2 hours prior to carbonation. [j] Color Test I was negative throughout the course of the reaction. [k] The mixture was stirred at room temperature for 48 hours after adding the benzyl chloride solution. [m] The mixture was stirred at −50° for one hour prior to carbonation.

ous layer was made acidic with hydrochloric acid and then extracted again with ether for 16 hours. After drying the ethereal solution over anhydrous sodium sulfate and then filtering off the sodium sulfate, the ether was distilled under reduced pressure; the resulting residue was an oil which possessed the odor of phenylacetic acid. The oil was dissolved in a minimum amount of water and filtered while hot. Upon cooling to 5° colorless plates crystallized out. Further concentration of the mother liquor yielded more of the same type of crystals. The total yield of product was 0.068 g., m.p. 76.5–78°. A mixed melting point with an authentic sample of phenylacetic acid (m.p. 77–78°) showed no depression.

The organic layer obtained after carbonation was dried over anhydrous sodium sulfate and then distilled under reduced pressure to remove the ether. Further distillation of the residue under reduced pressure yielded 2.9 g. (67.5%) of material melting at 51.5–53°. A mixed melting point of this material with an authentic sample of bibenzyl (m.p. 53°) showed no depression.

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE
AMES, IOWA

The Effect of Borate and Sulfate Ions upon the Electrophoretic Mobility of Mucoproteins[1]

BY E. GOLDWASSER AND M. B. MATHEWS[2]

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During a study of the physical properties of the carbohydrate-containing protein from human plasma that is an inhibitor of hyaluronidase,[3] we noted an appreciable increase in electrophoretic mobility when the buffer contained borate or sulfate ions. It seemed plausible to assume that the mobility increase was due to a similar complex with the carbohydrate moiety of the mucoprotein because of the well known ability of borate to form charged complexes with a variety of carbohydrates. The action of sulfate remained unexplained. These studies were thus extended to include bovine serum albumin as well as another serum mucoprotein.[4]

Experimental.—Crystalline bovine serum albumin was obtained from Armour and Co. Dr. R. J. Winzler of the University of Illinois, generously supplied a sample of the purified serum mucoprotein, orosomucoid, while the serum inhibitor of hyaluronidase was isolated from human serum in earlier experiments.[3] Electrophoresis was carried out in buffers of ionic strength 0.1 and *p*H 8.6, with only the composition altered (Table I), in a Spinco model H electrophoresis apparatus, with standard 11-ml. cells at 1° and at field strengths of 4.5 to 5.2 volts/cm. for serum albumin and orosomucoid. The hyaluronidase inhibitor was run in a semi-micro cell (2 ml.) at a field strength of 0.75 volt/cm. The results are summarized in Table II.

TABLE I

COMPOSITION OF BUFFERS, 0.1 μ AND *p*H 8.6

Component	Veronal–borate, *M*	Veronal–sulfate, *M*	Borate–sulfate, *M*
Veronal	0.011	0.0037	...
Sod. veronal	.075	.025	...
Boric acid	.086	....	0.086
Sod. borate	.025	....	.025
Sod. sulfate	...	.025	.025

TABLE II

DESCENDING MOBILITIES ($\times 10^5$ CM.^{2} VOLT^{-1} SEC.$^{-1}$) IN VARIOUS BUFFERS AND RATIO TO MOBILITY IN VERONAL BUFFER

Buffer	Albumin[a] Mobility	Albumin[a] Ratio	Orosomucoid[a] Mobility	Orosomucoid[a] Ratio	Serum inhibitor[b] Mobility	Serum inhibitor[b] Ratio
Veronal	−6.7	1.0	−5.2	1.0	− 5.4	1.0
Borate–sulfate	−8.1	1.2	−7.1	1.4	−10.2	1.9
Veronal–borate	−6.6	1.0	−6.2	1.2	− 7.5	1.4
Veronal–sulfate	−8.0	1.2	−6.8	1.3	....	..

[a] Concentration was 5 mg./ml. [b] Concentration was 3 mg./ml.

(1) This research was supported in part by a grant from the Chicago Heart Association.

(2) Work done during tenure of an established investigatorship of the American Heart Association.

(3) G. Berenson, J. Newman, M. B. Mathews, E. Goldwasser and A. Dorfman, *J. Biol. Chem.*, in press.

(4) R. J. Winzler, A. W. Devor, J. W. Mehl and I. M. Smyth, *J. Clin., Invest.*, **27**, 609 (1948).

The data indicate clearly that the presence of borate ion in the buffer has no effect upon the mobility of serum albumin, while it increases the mobility of orosomucoid about 20% and that of the inhibitor about 40%. On the other hand, quite unexpectedly, the presence of sulfate ion in the buffer has a substantial effect upon the mobilities of all proteins. As far as we are aware, no other studies of the binding of sulfate ions by proteins have been published. Using the charge-mobility relationship of Longsworth and Jacobsen,[5] we estimate that a minimum of about 3 moles of sulfate ion is bound per mole of serum albumin. Dialysis equilibrium studies in which methyl orange was used as a competing ion, do not reveal appreciable binding of sulfate by albumin. However, this method may be too insensitive.[6]

Paper electrophoresis studies[7] have shown that mono- and oligosaccharide derivatives combine strongly with borate when a glycosyl hydroxyl group and an adjacent hydroxyl group are free. However, union of borate with carbohydrate can also occur, though less strongly, when vicinal hydroxyl groups only are available.

In a recent report, Northcote[8] has described the free solution electrophoresis in borate buffer, *p*H 9.2, of neutral polysaccharides and has concluded that complexing with borate occurs with both *trans*- and *cis*-vicinal hydroxyl groups, although more strongly with *cis*- than with *trans*-hydroxyls. Since the comparisons were not made in buffers of the same ionic strength, this conclusion has not been clearly established.

The above data extend these observations to carbohydrate containing proteins and suggest that the carbohydrate moiety of the molecule possesses vicinal hydroxyl groups available for complexing with borate ion. In the case of the inhibitor, such complexing occurs without detectable denaturation.[3] Electrophoresis in borate containing solutions appears to be a procedure of value for electrophoretic separations of mucoproteins. In addition, information as to structure may also be obtained.

(5) L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid Chem.*, **53**, 126 (1949).

(6) I. Klotz, personal communication.

(7) A. B. Foster and M. Stacey, *J. Appl. Chem.*, **3**, 19 (1953).

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ARGONNE CANCER RESEARCH HOSPITAL AND
DEPARTMENTS OF BIOCHEMISTRY AND PEDIATRICS
THE UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

Formation of 3,5-Dinitrobenzoates from Acetals and Ketals with 3,5-Dinitrobenzoyl Chloride[1]

BY OLIVER GRUMMITT AND JAMES A. STEARNS, JR.

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During some work on a commercial solvent we observed what appeared to be the direct formation of methyl 3,5-dinitrobenzoate from dimethyl acetal and 3,5-dinitrobenzoyl chloride. This suggested the possibility of characterizing the alcohol groups of acetals and ketals as solid 3,5-dinitrobenzoates without preliminary hydrolysis and separation of the alcohol.[2] Earlier, Post[3] had reported 60–70% yields of alkyl benzoates from equimolar quantities of aliphatic acetals and benzoyl chloride allowed to react at reflux temperatures. The second product was believed to be an α-chloroalkyl alkyl ether. Thus, with diethyl acetal the reaction would be

$$CH_3CH(OC_2H_5)_2 + C_6H_5COCl \longrightarrow C_2H_5OOCC_6H_5 + CH_3CH(Cl)OC_2H_5 \quad (1)$$

Previously, zinc iodide had been used to catalyze the similar reaction of dimethyl acetal and benzoyl chloride,[4] but no catalyst is needed.[3] Therefore, the replacement of a single alkoxy group in the acetals by chlorine from benzoyl chloride takes place more readily than the analogous reaction of dialkyl ethers with acid chlorides which requires zinc or ferric chloride.[5]

Ketals of methyl ketones react with benzoyl chloride at 100–140° to give a benzoate ester and, instead of an α-chloroether, an alkyl chloride and the methyl ketone are formed[6]

$$CH_3C(OCH_3)_2R + C_6H_5COCl \longrightarrow CH_3OOCC_6H_5 + CH_3Cl + CH_3COR \quad (2)$$

Esters also have been obtained from acetals and ketals with acids or acid anhydrides but vigorous conditions were required.[7–9] With an acid catalyst, however, methylal and aliphatic acid anhydrides (not aromatic acid anhydrides) at reflux give 86–95% yields of methoxymethyl acetate, etc.[10]

In studying the formation of 3,5-dinitrobenzoates from acetals and ketals with 3,5-dinitrobenzoyl chloride, we considered reactions 1 and 2, and also the possibility that both alkoxy groups might be replaced by chlorine with the formation of an alkylidene chloride, a vinyl chloride or an acetylene, depending upon the extent of dehydrochlorination

$$RCH_2CH(OR')_2 + 2ArCOCl \longrightarrow \begin{array}{l} RCH_2CHCl_2 + 2ArCOOR' \quad (3) \\ \downarrow \\ RCH{=}CHCl + HCl \\ \downarrow \\ RC{\equiv}CH + HCl \end{array}$$

The stoichiometry of the reaction of anhydrous 1,1-dimethoxyethane and 3,5-dinitrobenzoyl chloride was determined at several reactant ratios, times and temperatures. With excess acetal, methyl 3,5-dinitrobenzoate is not formed until the reaction time at reflux (*ca.* 70°) exceeds five minutes. At a 5.5/1 mole ratio and 30 minutes reflux the yield of ester is 53%. Reversing the reactant ratio to excess acid chloride gave mixtures which decomposed at 65°, but at room temperature for 72–120 hours the yields were 45–50%. Thus, under these conditions the ester readily forms through

(1) From the M.S. thesis of J. A. S., Western Reserve University, January, 1953.

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