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(α-D-GLUCOPYRANOSYLURONIC ACID) (α-D-GLUCOPYRANOSIDURONIC ACID) AND SIMPLE DERIVATIVES

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ABSTRACT

For preparing pseudo cord-factors, similar in gross structure to α,α-trehalose 6,6′-dimycolate, we have synthesized (α-D-glucopyranosyluronic acid) (α-D-glucopyranosiduronic acid) ("trehalose dicarboxylic acid") by catalytic oxidation of trehalose. Such simple derivatives as the dimethyl ester and the hexa-*O*-acetyl diacid chloride are useful for the attachment of lipid substituents to the carbohydrate core to yield the desired pseudo cord-factors. Some of the glycolipids have toxic and antitumor properties resembling those of the natural product.

INTRODUCTION

The diverse biological activities of cord factor (α,α-trehalose 6,6′-dimycolate, **1**), provide it with an almost unrivalled importance amongst mycobacterial lipids[1–4]. It is peculiarly toxic for mice[1,2,5,6]. On the one hand, **1** is toxic for macrophages in culture[7], while on the other it can stimulate peritoneal macrophages[8]. In fresh plasma, it behaves as a chemotaxigen for macrophages[9], and itself has chemotactic properties[10]. Possibly because of their granulomagenic[11,12] and also adjuvant properties[13–15], trehalose dimycolates have emerged as a principal factor in contributing to antitumor activity of a variety of substances in themselves much less active (or even inactive) in the absence of **1** (or closely related analogs[16–19]).

Even before the structure of cord factor was firmly established, synthetic efforts were initiated to examine structure–biologic function relationships[20]. Recently, Kato and Asselineau have examined the influence upon biological activities of the stereochemistry of the carbohydrate moiety of cord-factor analogs, namely of 6-mycolates of methyl D-glucosides, -mannoside, -alloside, -galactoside, and of D-glucitol. The results revealed "the importance of the configurations at the asymmetric centers and (suggested) the necessity of a definite conformation of the polar moiety of the molecule"[21].

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Simpler 6,6′-diesters of α,α-trehalose (which are more-genuine analogs of the cord-factor structure) have also been extensively studied[22–24]. We have sought instead to examine the influence of structural variations in the groups linking the carbohydrate core and the lipid substituents, in order to test the simplistic assumption that some of the biological activities may depend principally upon the amphipathic (and probably therefore surface-active) properties conferred by a polar core-moiety substituted with sufficiently large, lipophilic groups. Our present studies are targeted at synthesis of "pseudo cord-factors" in which the 6-hydroxymethyl groups of trehalose have been oxidized to carboxylic groups. To these reactive groups, lipid substituents may be attached by either ester or amide linkages, and we have prepared representatives of both classes. This communication describes the synthesis of the key intermediate, (α-D-glucopyranosyluronic acid) (α-D-glucopyranosiduronic acid) (**4**) and of simple derivatives.

DISCUSSION

Respecting cord factor (**1**), transposition of the 6-hydroxymethyl groups of the carbohydrate (to the 6-carboxylic acids) and the lipid carboxylic acid (to the lipid alcohol) would afford what we term "mirror" pseudo cord-factor (**2**), whereas conjugation of the carbohydrate dicarboxylic acid to the lipid moiety via an amide linkage gives rise to a "mirror amide" type of pseudo cord-factor (**3**). Both classes are derivatives of "trehalose dicarboxylic acid" (**4**).

The diacid **4** was obtained in satisfactory yield by platinum-catalyzed oxidation of α,α-trehalose in aqueous hydrogencarbonate with oxygen according to the general method of Mehltretter[25]. Oxidation of 2,3,4,2′,3′,4′-hexa-*O*-acetyl-α,α-trehalose under substantially anhydrous conditions with either permanganate, chromium trioxide, or sequences of these oxidants gave only indifferent results.

Catalytic oxidation of α,α-trehalose (3–6 h) afforded a mixture (5 or 6 components, designated I–V in order of decreasing mobility, plus starting material) that was effectively resolved by paper chromatography. Fig. 1 shows the distribution of products obtained after 1, 2, and 3 h of oxidation. The most mobile component was residual trehalose. The mixture was preparatively separated by multiple chromatography on a column of powdered cellulose.

Component III, first isolated by preparative paper-chromatography, was purified to homogeneity and identified as the monocarboxylic acid, (α-D-glucopyranosyluronic acid) α-D-glucopyranoside; it comprised ~35% of the oxidation products, but its recovery was not rigorously quantitated; nor was it studied in detail. It is enriched in the early column effluents, and is the principal contaminant in the first effluents containing component V. It was identified by its neutralization equivalent and by elementary analysis of its potassium salt; on prolonged hydrolysis in M hydrochloric acid it afforded glucose, glucuronic acid, and a degradation product obtained from the latter under identical conditions.

Component V, obtained from the penultimate column effluents, was the desired

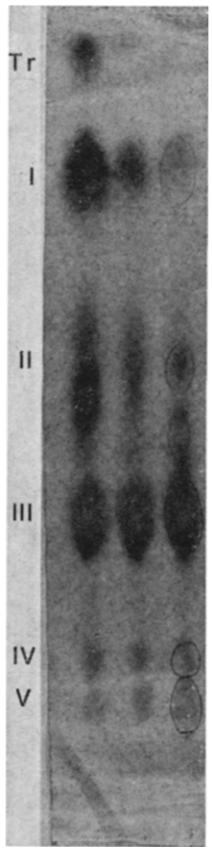
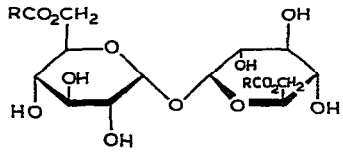


Fig. 1. Paper chromatogram of products from catalytic oxidation of α,α-trehalose. Samples, left to right, were taken at 1-, 2-, and 3-h intervals. Origin at bottom: descending development for 40 h with 5:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water. From top: Tr (trehalose); components, I, II, III (α-D-glucopyranosyluronic acid α-D-glucopyranoside), IV, and V (compound **4**). Components I, II, and IV are as yet unidentified.

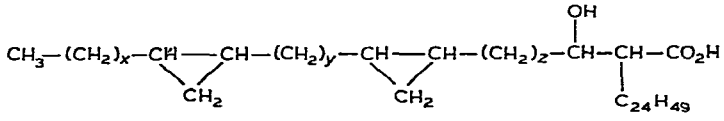
dicarboxylic acid (**4**); it comprised ~40% of the crude products after 5–6 h of oxidation. In the primary column chromatography of the oxidation-product mixtures, concentrates of component V judged to contain a maximum of ~95% **4** were obtained as major fractions. Rechromatography gave homogeneous **4** in ~30% overall yield, the remainder being distributed in still-contaminated fractions. Components I, II, and IV have not as yet been identified. As they reduce alkaline silver nitrate at once (unlike components III and V), it is possible that some of these may correspond to the aldehyde precursors of the latter. Component IV is cleanly separated from **4** by column chromatography.

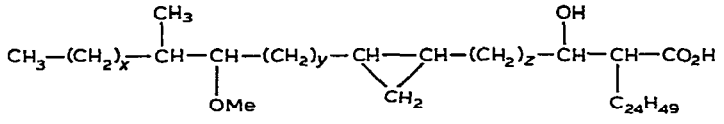
The diacid **4** was characterized by chromatographic patterns, optical rotation, i.r. and 100-MHz ^{1}H-n.m.r. spectrometry, and identified by hydrolysis to glucuronic acid, by elemental analysis, neutralization equivalent, and by reconversion into α,α-trehalose by reduction of the acid or of its dimethyl ester (**5**). The latter was readily



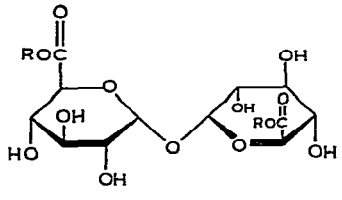
1 RCO_2H = Mycolic acids

Representative examples:





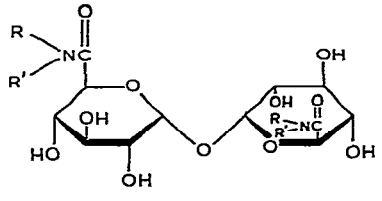
In general *x*, *y*, and *z* are odd, even, odd; for example 17.14.17



2

"Mirror" pseudo cord factor

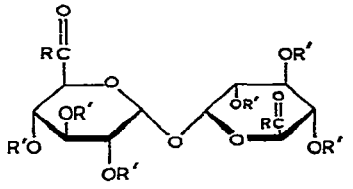
R = alkyl



3

"Mirror amide" pseudo cord factor

R = alkyl; R' = alkyl or H



4 R = OH, R' = H "Trehalose dicarboxylic acid"

5 R = OMe, R' = H

6 R = OH, R' = Ac

7 R = OMe, R' = Ac

8 R = Cl, R' = Ac

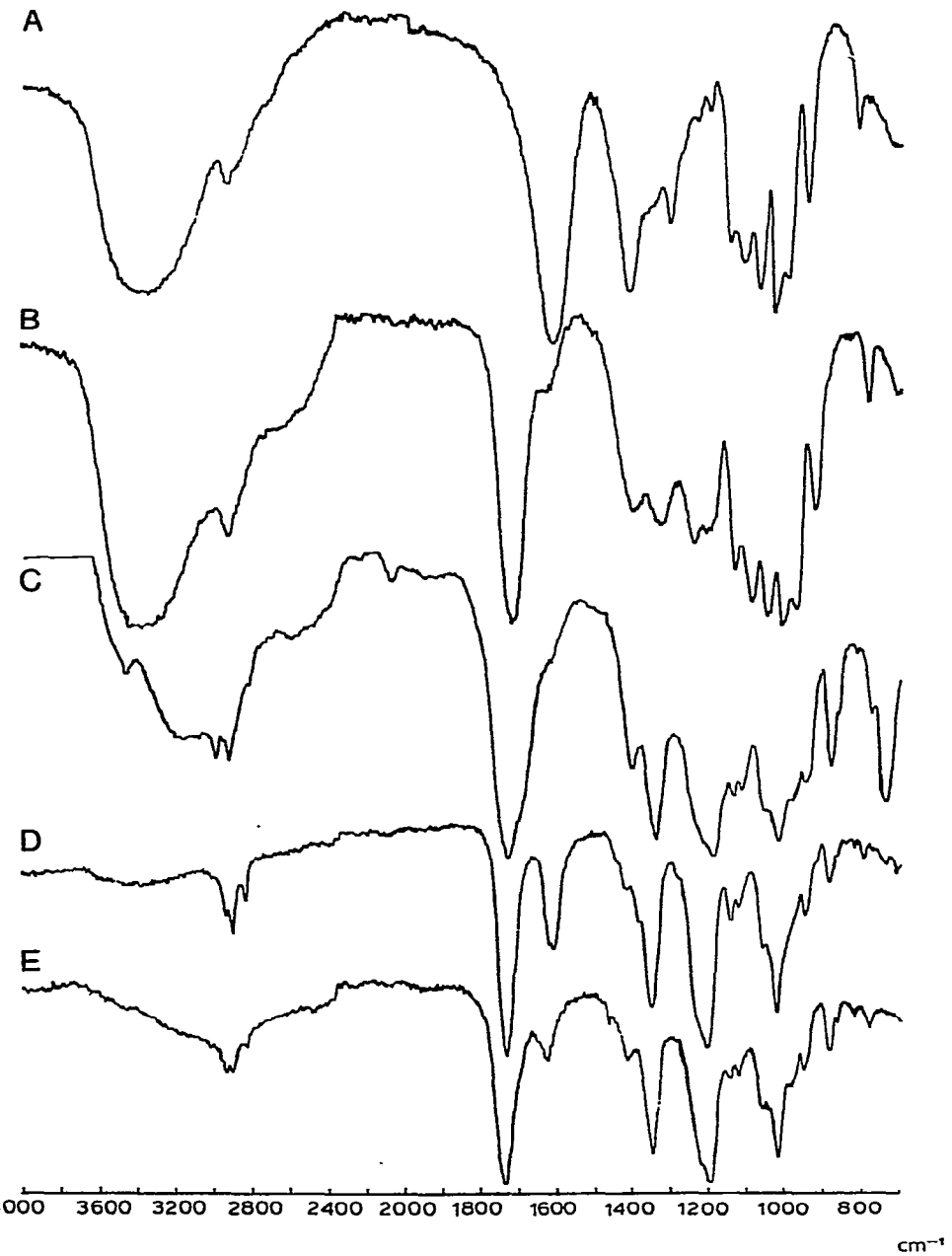


Fig. 2. Infrared spectra of (A) compound **4**, dipotassium salt; (B) compound **4**, free acid; (C) compound **6**; (D) disodium salt of **6**; (E) compound **8**. Carbonyl absorptions arising from carboxyl and/or ester are displaced to higher wave-numbers. In (E) there is no evidence of absorption at 1800 cm^{-1}, but the carbonyl band at 1765 cm^{-1}, evidently caused by both acetate and acid chloride, is merely sharpened. The weak band at ~1630 cm^{-1} arises from a trace of residual *N*,*N*-dimethylformamide.

prepared by mild Fischer esterification in methanol–hydrogen chloride or with diazomethane. This product was also characterized by i.r., ^{1}H-n.m.r. and mass spectrometry, and by elemental analysis. It was a useful intermediate for preparing simple pseudo cord-factors. Because of its t.l.c. and g.l.c. (of the trimethylsilyl ether or hexaacetate) characteristics, it was also useful for comparing derivatives of **4** that were convertible into the dimethyl ester **5**.

The i.r. spectra of **4** and several of its simple derivatives display a curious bathochromic shift of the carboxyl absorption-band. Instead of the expected absorption at 1710 cm^{-1}, the free-acid form of **4** gave a spectrum (Fig. 2B) showing broad carboxyl absorption, centered at ~1745 cm^{-1}. Most esters of **4**, whether of the hydroxyl groups or of the 6-carboxyl groups, showed a similarly displaced i.r. pattern (with a peak at about 1765 cm^{-1}), so that contributions of the free carboxylic acids could be recognized only through their conversion into salts. (compare Fig. 2A, C, and D). Other aberrations in the i.r. spectra of derivatives of **4** are alluded to later.

Attempts to peracetylate **4** with acetic anhydride–pyridine, or with acetic anhydride in the presence of either sodium acetate or zinc chloride, or with acetyl chloride invariably led to mixtures. Some of these may have resulted from partial lactonization (i.r. evidence), and other components were probably acetylated, mixed anhydrides of **4** and acetic acid; some of the products may have had both features. Rapid acetylation was achieved when the free acid form of **4** was dissolved (or suspended) in acetic anhydride and a trace of conc. sulfuric acid added. The product (**6**) was generally obtained homogeneous, but occasionally a (presumably) lactonized product(s) was obtained, as indicated by i.r. spectroscopy and by titration with alkali.

The i.r. and n.m.r. characteristics of the dicarboxylic acid hexaacetate **6**, and its neutralization and saponification equivalent and elemental analysis, all are in accord with the assigned structure; diazomethane converted it into the dimethyl ester (**7**), identical with the product prepared by acetylation of compound **5**.

The mass spectrum of **7** confirms the assigned structure: the diester hexaacetate would be expected[26–29] to give a primary oxonium ion having *m/e* 317. This ion successively loses molecules of acetic acid, ketene, and acetic acid, to give the series *m/e* 317, 257, 215 and 155. These ions are the only prominent peaks in the spectrum, except for *m/e* 42 (ketene); the molecular ion is absent.

Treatment of the hexaacetate with thionyl chloride, preferably in the presence of *N,N*-dimethylformamide[30] leads to a reactive product tentatively formulated as the acid chloride **8**. Its structure was deduced from its reactivity as an acylating agent for alcohols and amines[31], but its i.r. spectrum (Fig. 2) does not show the expected absorption at ~1800 cm^{-1}; instead the peak is observed (as with most derivatives of **4**) at 1765 cm^{-1}, but is sharpened as compared with similar absorption-bands in **4** and **6**. This presumed acid chloride reacts immediately with methanol to yield the dimethyl ester hexaacetate **7**. The acid chloride **8** is hydrolyzed very readily.

As described in companion communications[31], compound **8** satisfactorily acylates secondary amines and alcohols of higher molecular weight to give acetylated pseudo cord-factors.

EXPERIMENTAL

General methods. — Solutions were evaporated *in vacuo*. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

Catalytic oxidation of α,α-trehalose: (*α-D-glucopyranosyluronic acid*) (*α-D-glucopyranosiduronic acid*) (**4**). — To α,α-trehalose dihydrate (2.0 g) in water (80 mL), 0.4 g of pre-reduced platinum dioxide was added, the mixture was stirred vigorously at 65°, and sparged for 5 h with oxygen dispersed through a sintered-glass tube. Sodium hydrogencarbonate (1.1 g) was added from time to time to maintain the mixture slightly alkaline. The cooled suspension was filtered and the filtrate passed through a bed of AG-50 W-X8 ion-exchange resin (H^+ form). The effluent was made neutral with M potassium hydroxide, and evaporated to dryness; yield 2.4 g. Paper chromatography (compare Fig. 1) indicated five components (I–V), in addition to some residual starting material.

The mixed oxidation products (1 g) were dissolved in water (2 mL) and loaded onto a column of powdered cellulose (5.7 × 45 cm) that had been pre-conditioned with the eluent (4:1 ethanol–water) used throughout the chromatography. Fractions (8 mL) were collected after V_o = 750 mL had passed through the column. Following fraction 210, 19-mL fractions were subsequently collected. Fractions 90–130 contained almost pure component III (the monocarboxylic acid), whereas the dicarboxylic acid **4** (component V, Fig. 1) began to emerge, grossly contaminated, at fraction 210. Almost pure **4** was isolated from fractions 370–470. The results concerning component V are summarized in Table I.

Homogeneous **4** was obtained from fractions (c–e of Table I) by rechromatography on a cellulose column employing the same eluent (yield, ~30%). The purified dipotassium salt of **4** was obtained as a powder from concentrated aqueous solutions of the salt by precipitation with an excess of methanol. Drying at room temperature *in vacuo* over phosphorus pentaoxide gave the monohydrate; it sintered and foamed at 140° and slowly decomposed without a distinct m.p.; $[\alpha]_D^{25}$ +123° (*c* 2.0, water); n.m.r. (D_2O): δ 5.26 (d, $J_{1,2}$ 3.3 Hz, H-1,1′), 4.44 (d, $J_{4,5}$ 9.6 Hz, H-5,5′), and 3.8 (m).

TABLE I

RECOVERY OF DIACID **4** FROM OXIDATION MIXTURE[a]

Fractions	*Weight (mg)*	*Estimated content of* **4**	
		%	*Wt. (mg)*
a 211–305	117	15	17
b 306–325	21	25	5
c 326–370	120	70	84
d 371–480	487	95	463
e 481–520	99	60	60

[a]From 1 g of oxidation mixture. For details, see text.

The potassium salt was percolated through AG50WX-8 (H^+) resin and the effluent titrated with standard base, and the molecular weight of the salt was determined to be 444.0 (calc. 464.5).

Anal. Calc. for $C_{12}H_{16}K_2O_{13} \cdot H_2O$: C, 31.03; H, 3.96. Found: C, 30.81; H, 4.06.

The free-acid form of **4** was prepared from a solution of the dipotassium salt (143 mg) in water (1 mL) that was percolated through a column (1.1 cm × 13.5 cm) of AG50WX-8 resin (30–50 mesh, H^+ form). The column was washed with water (50 mL) and the effluent evaporated to dryness below 30° and the residue was dried further *in vacuo* over phosphorus pentaoxide; yield 115 mg (96%). The residue was extracted with anhydrous methanol, the mixture filtered, and the filtrate evaporated. The residue was redissolved in methanol (1 mL) and the diacid **4** precipitated as a powder by adding 2–3 mL of anhydrous ethyl acetate. The colorless product was filtered off, washed with ethyl acetate, and dried *in vacuo* over phosphorus pentaoxide at room temperature yield; 98 mg (82%). The dried product caramelized and melted indistinctly from 180 to >220°.

Anal. Calc. for $C_{12}H_{18}O_{13} \cdot H_2O$: C, 37.12; H, 5.19. Found: C, 37.04; H, 5.28.

Compound **4** is extremely hygroscopic and avidly absorbs moisture during filtration unless it is quickly transferred to a container for vacuum drying. In t.l.c. (silica gel plates developed with 5:1:3:1 1-propanol–ethylacetate–water–ammonia or in descending paper chromatography with the same solvent-mixture, it gave a single spot having the same mobility as the dipotassium salt. For reasons that are not clear (but which may depend upon a trace of mineral acid being present during isolation from the ion-exchanger effluent, and/or somewhat elevated temperature), a product was occasionally obtained that gave three spots in chromatography, the minor one having the mobility of the free acid (or dipotassium salt), whereas the major fractions, in about equal proportions, had higher mobility. Titration with standard base suggested that the mixture probably contains almost equal amounts of a mono- and a di-lactone. The (hydrolyzed) titration-mixture then gave a t.l.c. pattern like that of **4**, but recovery of solid material (as before) again gave a mixture of products.

[*(Methyl α-D-glucopyranosyl)uronate*] [*(methyl α-D-glucopyranosid)uronate*] (**5**). — The dicarboxylic acid **4** (92 mg) was dissolved in methanol (20 mL) made ~0.5M with anhydrous hydrogen chloride, the solution kept for 24 h at 23°, evaporated, and nitrogen blown onto the residue to remove residual hydrogen chloride. The product was dissolved in warm methanol (1mL) and precipitated at room temperature by slow addition of 15 mL of diethyl ether. Filtration, washing with ether, and drying *in vacuo* at 50° gave **5** as the hemihydrate, a white powder (84 mg, 87%). The material began to melt and foam at 121°, but resolidified and then melted with decomposition at 180–182°; $[\alpha]_D^{23}$ +167° (*c* 1.0, methanol); n.m.r. (CD_3OD): δ 5.10 (d, $J_{1,2}$ 3.7 Hz, H-1,1′), 4.46 (d, $J_{4,5}$ 9.0 Hz, H-5,5′), 3.55 (s, OCH_3), and ~3.7 (m). The integrals of H-1,1′; 5,5′; OCH_3; and H-2,2′, 3,3′, and 4,4′ were in the ratios of 1:1:3:3.

Anal. Calc. for $C_{14}H_{22}O_{13} \cdot 0.5\ H_2O$: C, 41.28; H, 5.69. Found: C, 41.23;

H, 5.94.

When the analytical sample was dried for an additional 4 h at 100°, it was obtained anhydrous.

Anal. Calc. for $C_{14}H_{22}O_{13}$: C, 42.21; H, 5.52. Found: C, 41.93; H, 5.69.

[*(2,3,4-Tri-O-acetyl-α-D-glucopyranosyl)uronic acid*] [*(2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronic acid*] (**6**). — The diacid **4** (97 mg) was dissolved–suspended in redistilled acetic anhydride (2 mL) and treated with ~1 μL of conc. sulfuric acid. During about 10 min of intermittent shaking, complete dissolution occurred, and the mixture was transferred onto crushed ice (5 g) and agitated until the excess of anhydride had disappeared. The product was extracted into 5 5-mL portions of ether, the combined extracts were washed once with a small amount of water (saturated with ether), and the ether solution was evaporated to dryness *in vacuo* to afford **6** as fine needles (93 mg). Extraction of the aqueous phases with chloroform afforded an additional 9 mg of **6** (yield 64%). It sintered at 120° and melted at 166–170°; $[\alpha]_D^{23}$ +151° (*c* 0.46, chloroform); n.m.r. ($CDCl_3$): δ ~5.35 (H-1–4 and 1′–4′), 4.41 (d, $J_{4,5}$ 9.8 Hz, H-5,5′), and 2.08 (s, acetyl). The integration of protons for H-1,1′,2,2′,3,3′,4,4′; H-5,5′; and acetates was in the expected ratio of 4:1:9. When dried *in vacuo* at 60°, the product was obtained as a monohydrate.

Anal. Calc. for $C_{24}H_{30}O_{19} \cdot H_2O$: C, 45.1; H, 5.03. Found: C, 45.5; H, 5.01.

Further drying for 4 h at 100° afforded the anhydrous form[32].

Compound **6** gave a single spot in t.l.c. (silica gel, 100:7:0.7 chloroform–methanol–acetic acid) and diazomethane converted it into the dimethyl ester hexaacetate **7**, also obtained by acetylation of the analytically pure dimethyl ester **5**. By gas chromatography on SE-30 (6 ft.) at 225°, both preparations gave single peaks at 13.5 min. When a sample of **6** was made neutral and titrated for acetate groups, 5.8 equiv. were determined (theoretical 6.0).

[*(2,3,4-Tri-O-acetyl-α-D-glucopyranosyl)uronoyl chloride*] [*(2,3,4-tri-O-acetyl-α-D-glucopyranosid)uronoyl chloride*] (**8**). — The acetylated dicarboxylic acid **6** (930 mg) was dissolved in benzene (15 mL) containing 60 μL of triethylamine and 1 drop of *N,N*-dimethylformamide, and the mixture was treated with purified thionyl chloride (0.4 mL) and kept overnight at room temperature. The solvent was evaporated off with dry nitrogen and, after drying *in vacuo*, the residue was extracted with benzene and the mixture filtered to remove insoluble triethylamine hydrochloride. I.r. spectrophotometry of a neat sample (Fig. 2E) exhibited a sharpened absorption band at ~1765 cm^{-1} as compared with the dicarboxylic acid (Fig. 2B), but there was no absorption at 1810 cm^{-1}. The broad hydroxyl-group absorption seen for the free acid had disappeared.

Treatment of compound **6** with diazomethane on the one hand and rapid reaction of the diacid chloride **8** with anhydrous methanol on the other converted both compounds into the dimethyl ester **7**. Compound **8** was not further characterized, but was used immediately in condensations with amines or alcohols[31].

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