β Elimination of Glycoside Monosaccharide from a 3-*O*-(2-Amino-2-deoxy-D-glucopyranosyl)serine. Evidence for an Intermediate in Glycoprotein Hydrolysis[1]

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The β elimination of glycoside carbohydrate from β-hydroxy amino acids during glycoprotein hydrolysis in basic media to produce α,β-unsaturated amino acid derivatives as reactive intermediates is verified with a model compound, 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-L-serine methyl ester, which was synthesized and definitively characterized. Simulation of a possible enzyme–substrate intermediate with decreased electron density on the amino acid nitrogen was effected by conducting base-catalyzed hydrolysis on the above *N*-2,4-dinitrophenylserine derivative. In dilute base under a variety of experimental conditions, elimination of the hexosamine intact and formation of *N*-2,4-dinitrophenyl-α-aminoacrylic acid occurred. The latter compound was isolated crystalline in 20% yield from the basic hydrolytic medium and its identity was affirmed by nmr spectroscopy.

Peptide-to-carbohydrate covalent bonds in cereal substances and mucous glycoprotein are being investigated to discern the metabolic pathways of these compounds.[2-14] Pigman[4] recently demonstrated that basic hydrolysis of mucoprotein from the oral–gastrointestinal tract (mucin) in the presence of sodium borohydride gave, as the cleavage reduction products from a postulated *O*-glycosylserine and *O*-glycosylthreonine, alanine and α-aminobutyric acid, respectively. Apparently, the hydrolytic intermediate of serine and threonine is an α,β-unsaturated amino acid produced by a β elimination. Such a β elimination has been postulated[4] as possibly being the common base-catalyzed hydrolytic pathway for β-hydroxy amino acid glycosides from glycoprotein.

The elimination of leaving groups β to a carboxylic acid ester is a well-documented reaction.[15] Riley confirmed that β-hydroxy amino acids are also subject to elimination in the presence of base[16] and affirmed the enamine nature of the *N*-substituted α-aminoacrylic acid elimination product by acid hydrolysis to pyruvic acid, which was then isolated as the crystalline 2,4-dinitrophenylhydrazone. A study of this α-aminoacrylic acid as a reactive intermediate in chymotrypsin action[17] has shed light on the facility of β elimination of *O*-substituted serine as established by Photaki[18] in even very weakly basic solutions (0.1 *N* sodium hydroxide or alkyl amine) and extended to the transformation of L-serine peptides to L-cysteine peptides.[19]

Jones and co-workers tested the base stability of an *O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-L-serine[20] and found this glycosidic linkage rather unreactive to alkali. The present work describes the use of an electron-withdrawing *N* substituent on the amino acid nitrogen, whose inductive effect on the nitrogen and the amino acid α carbon is stabilized by a variety of resonance forms. This effect should induce more facile elimination of the glycosyl group and perhaps simulate an electron delocalizing enzyme–substrate intermediate at the amino acid nitrogen.

Heidberg and co-workers[21] established a correlation between the geometrical structure and electronic configuration of nitro aromatic amines as prototypes of conjugated systems. The authors were able to exclude from nitroanilines a quinoidal nitronic acid structure with the amine hydrogen atom bonded to the nitro group and a double bond between the amino nitrogen and the adjacent ring-carbon atom. However, they concluded that the barrier energies of amino-nitrogen rotation are so large that the linkage between the amino nitrogen and the ring carbon should have considerable double bond character. This latter point indicates that the two other σ bonds of the amino-nitrogen substituents in 2,4-dinitroaniline derivatives are close to being symmetrical and coplanar to the benzene ring with one oxygen of the 2-nitro group hydrogen bonded to the amino proton. An *N*-2,4-dinitrophenyl derivative of serine should, therefore, enhance the inductive effect on the carbon α to the amino acid carboxyl group. Coincidentally, the nmr studies on these nitroanilines[21] provided a good basis of comparison for structure determination of the *N*-2,4-dinitrophenylserine glycoside synthesized here.

Improved synthesis of known starting materials as well as the Koenigs–Knorr-type glycosidation reaction have been reported. Thus, Horton has de-

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tailed an efficient synthesis of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (I),[22] and Lloyd and Roberts[23] as well as Hardy[24] have contributed improvements to the Koenigs–Knorr reaction. Slight alterations in Horton's[22] method of synthesis of I were employed (see Experimental Section) to permit purification of the glycosyl halide to chromatographic homogeneity with greater degree of reproducibility than was experienced with the unmodified procedure. Because no literature reference could be found for a specific synthesis or record of physical constants for *N*-2,4-dinitrophenyl-L-serine methyl ester (II), the experimental procedure of this paper records the method followed for the synthesis and characterization of II.

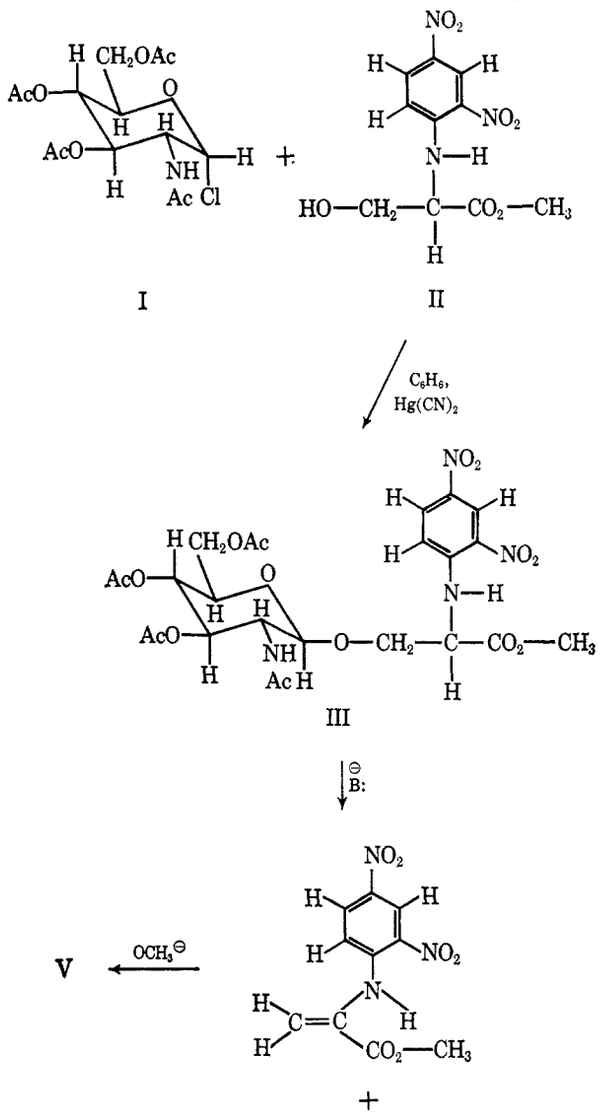
To compare the β anomer of an *O*-serine glycoside of 2-amino-2-deoxyglucose synthesized by Jones,[20] the β anomer of 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-L-serine methyl ester (III) was desired. Under the conditions employed in the glycosidation reaction, predominantly the β anomer of III would be expected.[25]

Characterization of III was carried out by acid hydrolysis of the glycoside into its component carbohydrate and amino acid fragments with chromatographic product identification, optical rotation, infrared spectrum, elemental analyses, and nmr spectrum. Thoroughness in structure determination was necessary because, if during the course of glycosidation a racemization of the amino acid should occur, four possible glycosides could result from attack on the intermediary carbonium ion at the anomeric carbon atom: D-hexose linked in both the α and β anomeric configurations to D- as well as L-serine. An amount of II alone, however, when subjected to the glycosidation conditions and work-up exhibited no change whatsoever in optical properties. A report that *N*-acylserine shows greater nucleophilicity than ethanol by three degrees of magnitude during acylation reactions of the serine hydroxy group[26] gave rise to the consideration that more of the α anomer than normally expected under these conditions might result. However, no abnormalities were noted in the reaction and a predominant single β-glycoside product resulted. Only a small amount of a second product, presumably the other anomer, was isolated. However, because of the admixture of this other glycoside with a side product of similar chromatographic mobility, this second glycoside has not been definitively characterized. The glycoside III crystallized from the reaction mixture in 17% yield without need of further resolution.

Recent articles on the nmr spectra of carbohydrate derivatives,[25,27–29] as well as a comprehensive review of the subject,[30] greatly facilitated interpretation of the nmr spectrum of III. The nmr results of this work for compounds II, III, and IV (see reaction sequence, Scheme I) are listed in Table I and compared with those for methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside as reported by Agahigian and co-workers[28] and *N*-methyl-2,4-dinitroaniline as reported by Heidberg.[21]

SCHEME I

GLYCOSIDE SYNTHESIS AND BASE-CATALYZED CLEAVAGE



The large coupling constant ($J_{1',2'}$ = 9 cps) for the anomeric proton resonance (τ 5.11) of III is indicative[30] of a 1′,2′-*trans*-diaxial situation of these protons. The chemical shift of the anomeric proton on this glycoside (III) is comparable with that of the methyl glycoside of Agahigian[27] in Table I (τ 5.35, $J_{1,2}$ = 8 cps), although the lower field positioning of the anomeric proton of III may be peculiar to a β-hydroxy amino acid glycoside. The size of the coupling constant of the anomeric protons of III in conjunction with the negative optical rotation of the compound ($[\alpha]^{25}D$ −37.60°, molecular rotation −23,106°)[31] suggest a β-D anomeric configuration for III.

The aniline N–H signal in II, III, and IV appeared in the low-field region assigned to this type of proton

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TABLE I. CHEMICAL SHIFTS AND COUPLING CONSTANTS FOR MODEL COMPOUNDS AND PRODUCTS[a]

Compound	Ar N–H	H–3″	H–5″	H–6″	H–2	H–3	H–1′	H–2′	H–3′	H–4′	H–5′	H–6′	–OCH₃	–OH	–OAc	–NAc	N–H	H₂C=	Unassigned signals
Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside[e]	...	...	...	...	...	...	5.35 (d, J = 8.0)	6.08	4.67	4.92	6.25	5.75	6.47	...	7.90, 7.94	8.03	3.88[b] (d, J = 8.5)	...	...
II	0.78	0.94 (0.91, 0.97, d, J = 3.0)	1.74 (1.63, 1.70, 1.78, 1.85, q, J = 9.0, 2.5)	3.13 (3.05, 3.21, d, J = 10)	5.58 (5.42, 5.50, 5.55, 5.62, 5.68, 5.75, st, J = 4.0)	5.89 (5.85, 5.93, d, J = 4)	...	...	...	...	...	...	6.18	7.66	...	...	...	...	...
III	0.78	0.94 (0.91, 0.97, d, J = 3.0)	1.74 (1.63 1.70, 1.78, 1.85, d, J = 9.0, 2.5)	3.13 (3.05, 3.21, d, J = 10)	5.52 (5.35, 5.43, 5.50, 5.50, 5.56, 5.60, 5.66, st, J = 4.0)	5.88 (5.85, 5.91, d, J = 3)	5.11 (5.05, 5.17, d, J = 9)	[b]	4.63 (4.48, 4.63, 4.80)	4.95 (4.80, 4.95, 5.12)	[b]	5.85 (5.81, 5.89, d, J = 3.5	6.23	...	7.94 7.98	8.03	3.95[c] (3.88, 4.02, d, J = 10)	...	5.91,[b] 6.31
IV	0.08[c]	0.85 (0.80, 0.89, d, J = 3.0)	1.74 (1.63, 1.70, 1.78, 1.85, q, J = 9.0)	2.56 (2.48, 2.63, d, J = 10)	See H_2C=	...	...	...	...	...	...	...	6.08	...	...	...	...	3.70 (3.68, 3.72, d, J = 2) 4.18 (4.16, 4.20, d, J = 2)	...
N-Methyl-2,4-dinitroaniline[21,d]		0.915 (0.89, 0.94, d, J = 2.6)	1.71 (1.60, 1.65, 1.76, 1.82, q, J = 9.7, 2.7)	3.06 (2.96, 3.15, d, J = 9.5)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

[a] Proton magnetic resonance, τ 10.00 with reference to tetramethylsilane internal reference: s, singlet; d, doublet; t, triplet, q, quartet; st, sextet. Coupling constant, J, in cycles per second. [b] Obscured by CH_2O– and –OCH_3 signals. As correlated with ref 27, these are in the H-2′ and H-5′ region. [c] Disappeared on deuteration. [d] Calculated from the cycles per second downfield shift from H_2CCl_2 reference, τ 4.67; see ref 21. [e] See ref 27.

by Heidberg.[21] Although the N–H of the aniline was poorly resolved as a much broader singlet peak than other signals, giving an integral of one proton, the methine proton of the serine moiety was sharply resolved into its symmetrical sextet. Using an analogy with Heidberg's work,[21] the downfield shift of aromatic protons in IV, *N*-2,4-dinitrophenyl-α-aminoacrylic acid methyl ester ($\tau_{H\text{-}3''}$ 0.85 and $\tau_{H\text{-}6''}$ 2.56 in IV *vs.* $\tau_{H\text{-}3''}$ 0.94 and $\tau_{H\text{-}6''}$ 3.13 in II and III), is possibly due to cross conjugation with the vinyl and carboxylate groups of the acrylic acid through delocalization of the 2s electron pair of the anilino nitrogen. A similar observation could be made about the deshielding of the N–H proton of IV ($\tau_{N\text{-}H}$ 0.08 of IV *vs.* $\tau_{N\text{-}H}$ 0.78 of II and III).

Treatment of III with mild base under various conditions gives 2-acetamido-2-deoxy-D-glucose and the α-aminoacrylic acid derivative, IV. A quantitative study of the kinetics of this reaction is presently underway. These studies indicate, however, that the β elimination of IV from III does occur even with the most mild base concentration in the cold. The elimination product, IV, was easily characterized by nmr and other means (see Experimental Section).

The "acid–base" compound (V) produced during the course of methoxide-catalyzed basic treatment of III could also be synthesized by further reaction of IV with methoxide. There is an analogy here with the work of Crampton on nitroanilino compounds.[32] Perhaps, as in Crampton's work, methoxide attack on the aromatic nucleus is taking place. The structure of V is being examined by nmr spectroscopy. Another structure from these syntheses still under investigation is that of the colorless hexosamine derivative isolated in reasonable yield from the glycosidation process used to synthesize III.

The *O*-blocking groups on III were retained during these hydrolytic studies to insure initial solubility in organic solvents when anhydrous conditions were desired. In point of fact, the de-*O*-acetylation of this glycoside proceeds more slowly than elimination, according to thin layer chromatographic examination of the reaction mixture. Only in the case of transamidation with *n*-propylamine were sufficient quantities of intact glycoside retained after de-*O*-acetylation. It is felt, therefore, that de-*O*-acetylation will probably be effected through use of amines under proper reaction conditions.

Experimental Section[33]

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl Chloride (I).—This compound was synthesized using a modification of the procedure of Horton.[22] 2-Acetamido-2-deoxy-D-glucose (17 g) was stirred in acetyl chloride (33 ml) without external heating using a condenser equipped with a drying tube (anhydrous calcium chloride). After an initial exothermic reaction, the mixture cooled and remained at room temperature with stirring until termination. Samples of the reaction mixture were spotted on silica gel G thin layer chromatography plates (solvent A, sulfuric acid location reagent), and the reaction progress was traced until completeness was indicated by maximum product (R_f 0.68, solvent A) formation and disappearance of starting material (2 days). After dissolving the mixture in dry dichloroethane (200 ml), the solution was shaken vigorously with a crushed ice–water slurry (500 ml) and then with cold, saturated sodium bicarbonate solution (150 ml). The organic layer was dried (anhydrous magnesium sulfate) and concentrated under diminished pressure to a volume of 70 ml, the solution was diluted with anhydrous ethyl ether (200 ml), and the crystals formed were separated from the supernatant by decantation. The damp, crude, brown crystalline product was washed three times with ethyl ether (25-ml portions), filtered, and dried under diminished pressure (8 mm) overnight: yield, 14.1 g (67%); mp 114° dec. Chromatography of this product on silica gel G (solvent A, sulfuric acid spray reagent) revealed two zones with R_f 0.68 and 0.32.

The crude product was taken up with warming in dry benzene and treated with decolorizing carbon, and the carbon was removed by filtration through a 10-mm thickness Celite[34] pad. Dilution of the benzene solution with anhydrous ether promoted crystallization of the purified product: 10 g, mp 123°, $[\alpha]^{25}D$ +115° (*c* 11, chloroform). Nmr spectroscopy data are in agreement with values reported by Horton.[25]

***N*-(2,4-Dinitrophenyl)-L-serine.**—Following the procedure of Sanger,[35] L-serine (3.78 g, 0.0358 mole) was dissolved in water (150 ml) to which solid sodium bicarbonate (12 g, 0.0645 mole) was added. 1-Fluoro-2,4-dinitrobenzene (12 g, 0.0645 mole) in 95% ethanol (300 ml) was slowly added to the L-serine mixture with shaking. The reaction was stirred and samples were withdrawn for inspection of reaction progress by thin layer chromatography on microcrystalline cellulose[33] (solvent B, ninhydrin spray reagent). The starting material, L-serine, disappeared after 2.5 hr, the mixture was concentrated under vacuum to assure complete removal of ethanol, and the cooled solution was neutralized with dilute hydrochloric acid (15 ml), whereupon a syrup precipitated which solidified to a partially amorphous yellow solid. The solid was filtered, washed with cold water, and dried: yield, 9.6 g (99%). A portion of this crude product was recrystallized from methanol–water: mp 167–170°; $[\alpha]^{25}D$ +9.8° (*c* 0.97, ethanol); λ_{max}^{KBr} 2.95 (OH, N–H), 3.4, 5.73 ($-CO_2H$), 6.15, 6.25 (Ar–), 6.54, 7.40 ($-NO_2$), 8.30, 9.82, 10.88, 12.00, 13.37, 13.95 μ.

***N*-(2,4-Dinitrophenyl)-L-serine Methyl Ester (II).**—Crude *N*-(2,4-dinitrophenyl)-L-serine (9.00 g, 0.0345 moles, above) dissolved in absolute methanol (120 ml) was treated in the cold (0°) with anhydrous hydrogen chloride until the solution was saturated. Estimation of product formation was carried out by thin layer chromatography on silica gel G[33] (solvent A, R_f of methyl ester, 0.80; R_f of starting free carboxy compound, 0.00), which indicated nearly completeness of reaction after 1.5 hr. The volume of methanol was reduced and the syrup was codistilled several times with 2-propanol to remove the last traces of hydrogen chloride. The syrup was taken up in 95% ethanol and filtered through a Celite[34] pad. Water was added to the yellow ethanol solution until turbidity appeared and the mixture was warmed until clear again. Cooling of the solution in a warm-water bath at the rate of bath equilibrium with room temperature (3 hr) gave crystals, which were filtered, washed with ether, and dried: yield, 6.8 g (69%); mp 99°; $[\alpha]^{25}D$ −26° (*c* 0.77, chloroform); λ_{max}^{KBr} 2.81 (OH), 2.93 (N–H), 3.50 (C–H), 5.70 ($-CO_2CH_3$), 6.15, 6.28 (Ar), 6.55, 6.65 ($-NO_2$), 7.00, 7.35 (NO_2),

(32) M. R. Crampton and V. Gold, *Chem. Commun.* (London), 256 (1965).

(33) Thin layer chromatography was carried out using the ascending technique on silica gel G (E. Merck Co., Darmstadt, West Germany; activated at 110° for 1 hr) with solvents 1:1 ethyl acetate–ether (solvent A), 3:1:1 1-butanol–acetic acid–water (solvent B), and 3:1 chloroform–acetone. Zones were located by spraying with sulfuric acid and heating the plate until charring occurred, with ninhydrin (0.2% in ethanol), with silver nitrate–sodium hydroxide spray reagent [W. E. Travelyan, D. P. Procter, and J. S. Harrison, *Nature*, **166**, 444 (1950)], aniline hydrogen phthalate spray [A. Schweiger, *J. Chromatog.*, **9**, 374 (1962)], or by observation of the yellow 2,4-dinitrophenyl derivatives. R_f refers to mobility of chromatographic zones with respect to the solvent front. Optical rotations were measured on a Rudolph manual polarimeter in a 2-dm tube. Melting points were determined on a National Instruments Co. "Melt Meter." Infrared spectra were measured on a Perkin-Elmer Model 137 Infracord infrared spectrophotometer with pellets pressed from a finely ground mixture of the sample with dried analytical grade potassium bromide. Nmr spectra were measured on a Varian Associates Model A-60 spectrometer in saturated deuteriochloroform solution with tetramethylsilane as internal reference. Sample solutions were not degassed prior to use. Deuteration exchange was carried out by shaking the deuteriochloroform solution with deuterium oxide. Microanalyses were determined by Dr. G. Weiler and Dr. F. B. Strauss, Oxford, England. Microcrystalline cellulose is Avicel, a product of the American Viscose Division of the Food Machinery Corp., Marcus Hook, Pa. See M. L. Wolfrom, D. L. Patin, and R. de Ledercremer, *J. Chromatog.*, **12**, 488 (1965).

(34) Celite is a product of the Johns-Manville Corp., Joliet, Ill.

(35) F. Sanger, *Biochem. J.*, **39**, 507 (1946).

8.19, 9.20, 10.80, 11.91, 13.40, 13.95 μ. Nmr data[33] are compiled in Table I.

Anal. Calcd for $C_{10}H_{11}N_3O_7$: C, 42.11; H, 3.89; N, 14.74. Found: C, 42.66; H, 3.88; N, 14.72.

Esterification was also effected with a stoichiometric amount of diazomethane in a tetrahydrofuran–ether mixture. The reaction was examined by thin layer chromatography using solvent A. Upon completion of reaction, the solution was evaporated to a syrup and dried several hours under diminished pressure. Crystallization was effected as above.

The product was chromatographically homogeneous in solvents A, B, and C.

3-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glycopyranosyl)-*N*-2,4-dinitrophenyl-L-serine Methyl Ester (III).—Mercuric cyanide (1.2 g, 0.00477 moles) was introduced into a solution of *N*-2,4-dinitrophenyl-L-serine methyl ester (II) (1 g, 0.00352 mole) in benzene (50 ml) contained in a round-bottom flask fitted with a side-arm distillation condenser and magnetic stirrer. The solution was heated with stirring until it boiled, and benzene (20 ml) was removed by distillation.[24] To this more concentrated solution was added 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (I) (1.2 g, 0.00324 mole) and additional benzene (5 ml) was removed by distillation. The reaction mixture was then fitted with a reflux condenser and heated to boiling. Progress of the reaction was followed by thin layer chromatography on silica gel G[33] (solvent A, sulfuric acid location reagent) at 15-min intervals until disappearance of I, R_f 0.68 (about 2 hr). After dilution of the reaction mixture with ethyl acetate (100 ml), the organic phase was washed successively with 1 *N* sodium chloride solution (twice, 75-ml portions) and then water (three times, 25-ml portions). The solution was dried (magnesium sulfate) and evaporated to a syrup. The syrup was washed with ether (20 ml) and cooled while under ether until the syrup solidified. The ether was decanted and the solid was dissolved in 95% ethanol (40 ml) with warming. Upon cooling the solution, long, yellow, thread-like crystals separated out and were collected by filtration. The product was washed on the filter with isopropyl ether. Two such work-ups of the syrup yielded 0.35 g (17%). Further small amounts of the same product were obtained from the mother liquor by preparative thin layer chromatography on silica gel G[33] (200 × 200 mm plates, solvent A). To extend elution time, Whatman No. 1 filter-paper wicks were hung over the back of the plate, pressed close to the adsorbent on the front by means of a 50 × 200 mm glass plate and held tightly with rubber bands. Yellow zones of the following R_f values were obtained in solvent A on silica gel G: 0.80 (*N*-2,4-dinitrophenylserine methyl ester), 0.34 (yellow product crystallized above), and 0.23, as well as a colorless zone (R_f 0.14), located by iodine vapor complex. After scraping the desired zones from the plates, the adsorbent was packed into a small column and eluted with ethyl acetate, and the eluate was concentrated to a syrup under diminished pressure. The zone with R_f 0.34 yielded a syrup which crystallized after the same procedure (above) was applied to the main reaction mixture syrup. In several preparative chromatographic runs the few milligrams of product isolated from the zone with R_f 0.34 were identical with that crystallized above: mp 176°; $[\alpha]^{25}D$ −37.6° (*c* 0.77, chloroform); λ_{max}^{KBr} 3.00 (N-H), 3.40 (C-H), 5.70 ($-CO_2CH_3$, -OAc), 6.02 (amide I), 6.15 (Ar–), 6.30 ($-NO_2$), 6.55 (amide II), 6.62 ($-NO_2$), 7.00, 7.30, 7.48 ($-NO_2$), 8.15, 9.50, 10.20, 10.80, 13.40 μ. Nmr spectroscopy data are tabulated in Table I.

Anal. Calcd for $C_{24}H_{30}N_4O_{15}$: C, 46.91; H, 4.92; N, 9.12. Found: C, 46.63; H, 4.80; N, 8.66.

The yellow syrup from the zone with R_f 0.23 on isolative thin layer chromatography was precipitated as an amorphous powder from isopropyl alcohol–isopropyl ether, but yield or other physical data are not available because the reaction mixture is still under chromatographic resolution (see discussion).

Colorless crystals were obtained (ethanol) from the zone with R_f 0.14 and are also under further investigation.

***N*-2,4-Dinitrophenyl-α-aminoacrylic Acid Methyl Ester (IV).**—An amount of III (0.17 g) in absolute methanol (20 ml) was treated with barium methoxide (0.3 *N*, 0.5 ml) at 0° under stirring, and the reaction course monitored by thin layer chromatography on silica gel G[33] (solvent A). After 40 min none of the reactant glycoside remained, and the base was neutralized by reaction with solid carbon dioxide. Examination of the reaction mixture by thin layer chromatography indicated zones with R_f 0.95 (yellow), 0.30 (colorless, charred with sulfuric acid spray, positive aniline hydrogen phthalate reaction, negative to ninhydrin spray), and 0.00 (yellow, did not char with sulfuric acid).

The solution was evaporated to near dryness at room temperature, benzene (15 ml) was added, and the insoluble yellow residue (V) was removed by centrifugation. After decanting the supernatant, the yellow solid was washed twice with benzene (10-ml portions), the washings were combined with the supernatant, and the solid was dried under vacuum: yield, 46.6 mg.

The yellow-orange solid (V) was taken up in water (8 ml) and hydrochloric acid (2 *N*, 2 drops) was added. The orange solution lost its color and a white precipitate appeared which was isolated by filtration: yield, 0.0201 g. Recrystallization from methanol gave 0.015 g, mp 205–206° dec, $[\alpha]^{25}D$ 0.00 (*c* 0.3, DMF).

Anal. Found: C, 45.38; H, 3.22; N, 17.81.

The supernatant liquid was evaporated to a small volume (8 ml) and applied to a Magnesol[36]–Celite[34] column (5:1, 50 g, column size 3 × 25 cm) which was developed with benzene–*t*-butyl alcohol (98:1, 600 ml). A fast-moving yellow zone was first eluted from the column. Removal of chromatography solvent from this first fraction, solution of the syrup in warm ethanol, and filtration, on cooling, permitted crystallization of orange needles: 0.015 g. (20.2%); mp 67–70°; $[\alpha]^{25}D$ 0.00 (*c* 0.2, $HCCl_3$); λ_{max}^{KBr} 3.00 (N–H), 5.75 ($-CO_2CH_3$), 6.15 ($H_2C{=}C<$), 6.28 (Ar–), 6.60 (NO_2), 6.91, 7.50, 8.20, 9.90, 10.48 ($_R{>}C{=}CH_2$), 10.80, 12.43, 13.50 μ. Nmr spectroscopy data are recorded in Table I.

Anal. Calcd for $C_{10}H_9N_3O_6$: C, 44.95; H, 3.40; N, 15.73. Found: C, 44.58, H, 3.53; N, 16.01.

The compound was chromatographically homogeneous in solvents A, B, and C: R_f 0.95 (solvent A, silica gel G[33]).

Elution of the extruded column cores (acetone) gave a solution which, on examination by thin layer chromatography (silica gel G, solvent A), indicated at least four minor zones oxidized by silver nitrate reagent. This acetone extract was evaporated to a syrup, the syrup was dissolved in anhydrous methanol (5 ml), the solution was cooled, and barium methoxide (0.3 *N*, 0.5 ml) was added with stirring. After 1 day in the cold, on chromatographic analysis (silica gel G, solvent B, silver nitrate spray reagent), a major zone was found from this reaction mixture with mobility identical with that of 2-acetamido-2-deoxy-D-glucose, R_f 0.20, and a second zone (presumably a degradation product) with greater mobility, R_f 0.31. The concentration of this latter component could be decreased by shortening the time of reaction mixture contact with base. No ninhydrin-active compounds were found among these chromatographic zones.

The glycoside III was also treated with sodium methoxide in methanol, yielding results identical with the barium methoxide treatment, as verified by chromatographic inspection under the same conditions used above.

Base-catalyzed methanolysis of the glycoside III in methanolic ammonia (saturated) was monitored by thin layer chromatography (solvent A) as the reaction proceeded in the cold. Colored and colorless zones similar to those described above appeared, though the rate of the reaction was slower in the formation of these components than with sodium or barium methoxide. After 1 day of reaction time, reddish orange crystals (a few milligrams) formed and were isolated by decanting the reaction mixture. These crystals were found to form a colorless solution in acid (hydrochloric acid) and yellow solution in base (sodium hydroxide). All zones in the chromatographic examination showed absence of ninhydrin-active functions.

Treatment of the glycoside III with Dowex-I exchange resin (OH^- form) at room temperature overnight in acetone–water solution (5:1) resulted first in absorption of the glycoside on the resin, cleavage of glycosidic linkage, and removal of the 2,4-dinitrophenyl blocking group. After thoroughly washing the resin with methanol–water (1:1), a ninhydrin-active zone with mobility identical with that of serine was discernible on chromatographic examination of the eluate.[37] The carbohydrate portion of the molecule was identified as several partially acetylated

(36) A product of Waverly Chemical Co., Marraneck, N. Y. Dry cleaners grade Magnesol was used in these studies.

(37) At the suggestion of one of the referees for this paper we speculate that upon elimination of IV from the glycoside, resin-catalyzed hydrolytic cleavage of the 2,4-dinitrophenyl group occurs, followed by a Michael addition of the elements of water to the unsubstituted α-aminoacrylic acid.

zones in the mixture (sulfuric acid spray, silica gel G,[33] solvent B).

The preceding experiment was also carried out using the carbonate form of Dowex-I anion exchange resin with identical results.

Use of *n*-propylamine as base at room temperature resulted in the cleavage of the glycoside to a similar 2,4-dinitrophenyl-α-aminoacrylic acid derivative described above (solvent A on silica gel G[33]) and a carbohydrate component as above. However, a yellow, slower moving zone component gave evidence of a certain amount de-*O*-acetylation without cleavage of the glycoside. This product is under further investigation.

With all the above conditions a selection of temperatures ranging from −30 to 50° were tried. Below −20° no glycoside cleavage was observed, whereas on visual estimation of products with thin layer chromatographic resolution, the base-catalyzed elimination was considerably accelerated at temperatures above room temperature.

The "acid–base" compound (V) described above could be synthesized by treating the isolated unsaturated *N*-2,4-dinitrophenyl-α-aminoacrylic acid methyl ester (IV) with barium or sodium methoxide in methanol.

Acid-catalyzed hydrolysis of the glycoside (III) in sulfuric acid (2 *N*) for 6 hr at 95°, neutralization with barium carbonate, and isolation of the hydrolysate by centrifuging gave a solution which was resolvable into two zones on thin layer chromatographic examination (silica gel G, solvent B) corresponding to 2-amino-2-deoxy-D-glucose and *N*-2,4-dinitrophenylserine (ninhydrin and silver nitrate location reagents[33]).

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Organic Sulfur Compounds. XVIII. Selective Addition of Thiols and Thiol Acids to Diallyl Maleate and Fumarate[1a]

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Aliphatic and aromatic thiols and thiolacetic acid were selectively added to the allylic double bonds of diallyl maleate and fumarate w th a free-radical, ultraviolet light initiated mechanism (at 16°). The "allylic" radical addition was mostly of anti-Markovnikov orientation, but minor amounts (up to 5%) of the Markovnikov-type (branched) allylic adducts were also formed. No isomerization of the maleic compound to the fumarate isomer occurred during these additions. The high specificity of the attack of thiyl radicals was attributed to their highly electrophilic nature. Base catalysis of the above reactions with triethylenediamine resulted in anionic Michael additions to the maleic or fumaric double bonds. These reactions were probably preceded by isomerization of the diallyl maleate to the fumarate, which indicates their reversibility.

In contrast to still widely held beliefs, free-radical reactions may show a remarkable selectivity. Earlier studies of free-radical addition reactions of thiol compounds to dienes and acetylenes in this laboratory[1b,2,3] showed that such reactions have a specific course which can be predicted on the basis of the structure of the starting olefin. Our more recent studies are concerned with selectively reacting certain groups in polyfunctional molecules while leaving others intact. In particular, this paper deals with selective free-radical additions of thiol compounds to the allylic double bonds of diallyl esters of maleic and fumaric acid. Anionic additions to the maleic and fumaric double bonds of the above compounds are also described.

Thiol compounds were a logical selection as model compounds for a study of both free-radical and ionic addition reactions of diallyl maleate and fumarate. In the presence of free-radical catalysts and often even in their absence, aliphatic and aromatic thiols are known to react in a clean, free-radical manner with olefinic double bonds. Base-catalyzed addition of thiols to maleic anhydride has also been described.[4,5] It was reasonable to assume that, under the proper conditions, thiols are especially likely to add to diallyl maleate by either a free-radical or an ionic mechanism and, therefore, are ideal reagents for testing the possibility of selective reactions. Although diallyl maleate and fumarate have been known for some time, none of their posssible selective addition reactions has been previously studied to our knowledge.

In this study mostly equimolar amounts of simple aliphatic and aromatic thiols, thiolacetic acid, and diethylphosphorodithioic acid were allowed to react with diallyl maleate and fumarate without solvent at ambient temperatures. The reaction mixtures were analyzed by a combination of n.m.r. spectroscopy and gas–liquid partition chromatography (g.l.p.c.). The adducts formed were isolated by fractional distillation *in vacuo*. N.m.r. spectra of the benzenethiol monoadducts are shown in Figure 1 for illustration of the distinctive spectral characteristics of the "allylic and maleic or fumaric adducts."

Results

Dependent on the reaction conditions, either the allylic or the maleic (fumaric) bonds of diallyl maleate (fumarate) could be selectively reacted.

Free-Radical Additions.—Methane-, ethane-, and benzenethiol could be selectively added under the effect of ultraviolet irradiation at 16° to the allylic double bonds of diallyl maleate and fumarate to form the corresponding anti-Markovnikov-type "allylic" mono- and diadducts (Tables I and II). No isomeri-

(1) (a) The contents of this paper were part of a presentation before the Organic Chemistry Division at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965, p. 47P. (b) A. A. Oswald, K. Griesbaum, W. A. Thaler, and B. E. Hudson, Jr., *J. Am. Chem. Soc.*, **84**, 3897 (1962).

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