amount which can be determined is about 0·5 μg of progesterone. The standard error expressed in extinction units was as high as 0·015. This value was found to be constant in the foregoing ranges of steroid quantities. This method was controlled radiometrically, using progesterone-4-^{14}C (The Radiochemical Centre, Amersham, England) and a current-flow gas counter; the loss of radioactivity during the procedure described did not exceed 5 per cent of the initial quantity added.

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Stability of *N*-Glycuronyl Amino-acids

In assessing the stability of the various possible linkages joining carbohydrates to amino-acid residues and in order to avoid base-catalysed acyl migration, we have synthesized *N*-(1,2 : 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycine and its β-DL-phenylalanine analogue and examined their behaviour on hydrolysis. The syntheses were effected by using as condensing agents either di*cyclo*hexylcarbodiimide[1], or tetraethylpyrophosphite[2].

1,2 : 3,4-Di-*O*-isopropylidene-D-galactose[3] was oxidized[4] with potassium permanganate and the crystalline potassium 1,2:3,4-di-*O*-isopropylidene-D-galacturonate obtained converted into the free acid by ethereal extraction of an acidified aqueous solution. The acid (21 g) was added to a stirred solution of freshly prepared benzylglycinate (13 g) in diethyl phosphite (50 ml.). Tetraethylpyrophosphite (22 g) was added and, after heating the stirred mixture at 100° for 10 min, it was poured into water. The precipitate was washed with saturated aqueous sodium hydrogen carbonate and recrystallized from ethanol to yield benzyl *N*-(1,2:3,4-di-*O*-isopropylidene-D-galacturonyl)-glycinate (20 g), m.p. 101°–102°, $[\alpha]_D$ −89·5° (*c*, 1·0 in chloroform) (found: C, 60·1; H, 6·6; N, 3·3. $C_{21}H_{27}NO_8$; requires C, 59·9; H, 6·4; N, 3·3 per cent).

Alternatively, glycine benzyl ester (1·39 g) was suspended in dry ether (100 ml.) and a mixture of di*cyclo*hexylcarbodiimide (3·1 g) and 1,2 : 3,4-di-*O*-iso-propylidene-D-galacturonic acid (3 g) added. After stirring at room temperature for 12 h, glacial acetic acid (3 drops) was added and the di*cyclo*hexylurea removed by filtration. The residue (4·99 g) after evaporation of the filtrate was crystallized from ether to yield benzyl *N*-(1,2 : 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycinate (1·21 g), m.p. 101°–102°, $[\alpha]_D$ −87·5° (*c*, 0·9 in chloroform), which was identical with the product described here. Debenzylation could be effected with sodium and liquid ammonia to yield *N*-(1,2 : 3,4-di-*O*-isopropylidene-D-galacturonyl)-glycine, m.p. 212° (from ethyl acetate), $[\alpha]_D$ −97·1° (*c*, 0·8 in chloroform) (found: C, 51·0; H, 6·5; N, 4·25. $C_{14}H_{21}NO_8$; requires C, 50·8; H, 6·3; N, 4·2 per cent).

In a similar way, β-DL-phenylalanine benzyl ester (2·8 g) in methylene chloride (100 ml.) was condensed with 1,2 : 3,4-di-*O*-isopropylidene-D-galacturonic acid (3·1 g) in the presence of di*cyclo*hexylcarbodiimide (3·5 g) to yield benzyl *N*-(1,2:3,4-di-*O*-isopropylidene-D-galacturonyl)-β-DL-phenylalanate (0·8 g), m.p. 115°–116° [from ethyl acetate–light petroleum (b.p. 60°–80°)], $[\alpha]_D$ −92·8° (*c* 0·88 in chloroform) (found: C, 65·7; H, 6·2; N, 3·0. $C_{28}H_{33}NO_8$; requires C, 65·7; H, 6·5; N, 2·7 per cent). Debenzylation afforded *N*-(1,2 : 3,4-di-*O*-isopropylidene-D-galacturonyl)-β-DL-phenylalanine with m.p. 211°–215° (from ethyl acetate–light petroleum (b.p. 60–80°)), and $[\alpha]_D$ −130·5° (*c*, 1·0 in chloroform) (found: C, 59·5; H, 6·6; N, 3·4. $C_{21}H_{27}NO_8$; requires C, 59·8; H, 6·45; N, 3·3 per cent).

The stability of the amide linkage in the two analogues was assessed by determination[5] of the hydrolytically released amino-acid with ninhydrin. Aliquots (2 ml.) containing *N*-(1,2:3,4-di-*O*-isopropylidene-D-galacturonyl)-glycine (144 μg) in N hydrochloric acid were hydrolysed to extents of 24·5, 42·9, 53·6 and 59·8 per cent after 1, 3, 4 and 6 h at 100°. The compound was more unstable in alkali and, after similar treatment with N sodium hydroxide at 100°, the percentage hydrolysis was 52·1 (0·5 h), 69·6 (2·5 h) and 83·7 per cent (3·5 h). By contrast, *N*-(1,2 : 3,4-di-*O*-isopropylidene-D-galacturonyl)-β-DL-phenylalanine was more unstable in acid than in alkali. Thus, aliquots (2 ml.) containing amino-acid derivative (1 mg) in 30 per cent aqueous alcoholic 0·5 N hydrochloric acid were hydrolysed to the following extents: 10 (1 h), 13 (2 h), 18 (3 h) and 35 per cent (6 h) at 92° compared with 4 (1 h), 11·5 (2 h), 24 per cent (4 h) in 30 per cent aqueous alcoholic 0·5 N sodium hydroxide at the same temperature.

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Influence of Hydrostatic Pressure on Orientation in Electrophilic Aromatic Substitutions

M. G. Gonikberg *et al.*[1–3] have recently found that at high pressures the attack of phenyl radicals on tertiary butyl benzene yields an appreciably higher proportion of the sterically strained 2-phenyl isomer than it does at atmospheric pressure.

We have observed a similar effect in the electrophilic nitration of 1,3-xylene by nitronium ions, NO_2^+. The nitrations were carried out under the same conditions and by the same methods that we had previously used in reaction rate measurements[4]. The initial composition of the reaction mixtures, expressed in mole fractions, was: nitric acid, 0·618; acetic acid, 0·369; 1,3-xylene, 0·013. We analysed the products by gas chromatography at several stages during the course of each reaction and found that the isomer ratios were independent of the extent of reaction. Table 1 lists the results.

Table 1. Mole Fractions of Isomers formed in the Nitration of 1,3-Xylene by Nitric Acid in Acetic Acid Solution at 0° C

Pressure (atm.)	Nitro isomer: 2-	4-	5-
1	0·107	0·880	0·013
800	0·150	0·833	0·017
1,200	0·164	0·816	0·020
2,000	0·171	0·829	

It will be seen that between 1 and 2,000 atmospheres the proportion of substitution at the sterically hindered 2-position increased by 60 per cent. The change is larger than that observed by Gonikberg[1–3] presumably because in 1,3-xylene there are two adjacent groups tending to obstruct attack on the 2-position. Our result supports Gonikberg's general thesis that an increase in pressure tends to favour the formation of sterically compressed isomers.