anion **8** was equal to the increase in quantum yield of the *N*-hydroxy lactam **10**.[20]

$$\Phi_{dec} = \Phi_{app}$$

Further investigation on the mechanism of this photoprocess including the electronic aspect of the excited anions is in progress.

(20) Quantum yields of the photoreaction of the anions **1**, **3**, **7**, and **12** were 0.1, 0.05, 0.02, and 0.01 in EtOH–EtONa, respectively.

(21) An authentic sample prepared according to the literature was identical in all aspects with **2**. Lewis, A. H. *Biochem. J.* **1927**, *20*, 1358.

(22) The hydroxamic acid **4** was identified by comparing with an authentic sample prepared by the method of Hauser et al. Hauser, C. R.; Renfrow, W. B., Jr. "Organic Syntheses"; Wiley: New York, 1943; Collect. Vol. II, p 67.

(23) 2-Ethoxynitrocyclopentane **5** was prepared as follows: 2-nitrocyclopentene (200 mg) was added to the solution of EtOH–EtONa (nitrocyclopentene:sodium = 1:1 in molar ratio) and stirred at room temperature. After 10 min of stirring, neutralization was carried out by 0.5 M HCl ethanolic solution followed by evaporation of ethanol and extraction by ether. Solvent removal from the extract gave a brown oil which was chromatographed on silica gel to yield **5** (65 mg), using benzene as an eluant. The following spectral data were obtained for this nitrocycloalkane **5**: NMR (CCl_4) δ 4.7 (1 H, sextet, J = 5 Hz), 4.3 (1 H, sextet, J = 5 Hz), 3.5 (2 H, q, J = 8 Hz), 2.3 (2 H, d, J = 8 Hz), 2.1–1.5 (4 H), and 1.2 (3 H, t, J = 8 Hz); IR (neat) 1550 (NO_2 asym) and 1370 cm^{-1} (NO_2 sym). This new compound gave satisfactory elemental analysis.

(24) Patchett, A. A.; Hoffman, F.; Giarrusso, F. F.; Schwam, H.; Arth, G. E. *J. Org. Chem.* **1962**, *27*, 3822.

A Micromethod for Determining the Branching Points in Oligosaccharides Based on Circular Dichroism

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As part of a new general approach for determining glycosidic linkages in oligosaccharides currently being developed in our laboratory, we describe a micromethod based on split CD curves of pyranose polybenzoates[1] which is suited for determining *glycosidic linkages at the branching points*. Despite the dramatic advancements made in structural analyses of polypeptides[2] and nucleic acids,[3] the complexity of oligosaccharide structures[4] has hampered the development of new microtechniques for structure determination. Although the sequencing of sugar units in oligosaccharides can be carried out by FD–MS,[5] the glycosidic linkage determination involves exhaustive methylation, hydrolysis and GC comparison of the monomeric methylated methyl glycosides with authentic specimens;[6] this identification process is severely restricted by the availability of standard samples. The method based on ^{13}C glycosidation shifts[7] is efficient and reliable but is applicable neither to samples of restricted supply nor to large molecules because of the appearance of ^{13}C NMR peaks in a narrow range.

A systematic investigation of more than 40 pyranose di-, tri-, and tetra-*p*-bromobenzoates clarified two aspects:[8]

(1) (a) Harada, N.; Nakanishi, K. *Acc. Chem. Res.* **1972**, *5*, 257. (b) Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy—Exciton Coupling in Organic and Bioorganic Stereochemistry"; University Science Books: Mill Valley, CA, in press.

(2) (a) Morris, H. R. *Nature (London)* **1980**, *286*, 447. (b) Biemann, K. In "Biochemical Applications of Mass Spectrometry"; Walter, G. R., Dermer, O. C., Eds.; Wiley: New York, 1980; Suppl. Vol. 1, p 469.

(3) (a) Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560. (b) Sanger, F.; Nicklen, S.; Coulson, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5463.

(4) Three monomers XYZ give rise to 6 isomeric tripeptides; however, 1056 isomeric trisaccharides are conceivable: Clamp, J. *Biochem. Soc. Symp.* **1974**, *40*, 3. (As quoted by: Sharon, N.; Lis, H. *Chem. Eng. News* **1981**, *March 30*, 21.)

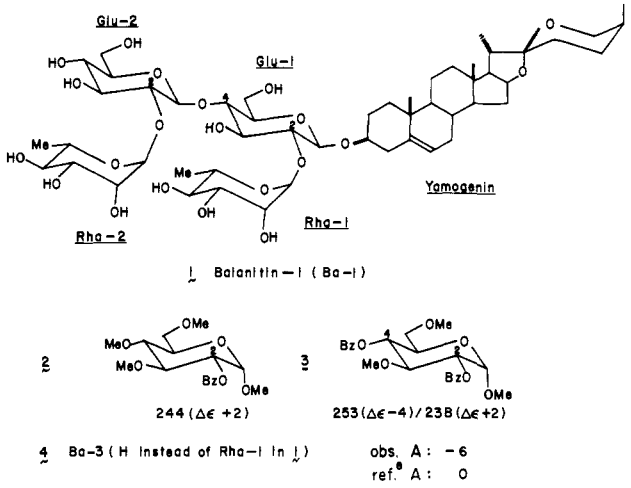
(5) Schulten, H. R.; Komori, T.; Kowasaki, T. *Tetrahedron* **1977**, *33*, 2595.

(6) Bouveng, H. O.; Lindberg, B. *Adv. Carbohydr. Chem.* **1960**, *15*, 53.

(7) (a) Kasai, R.; Suzuo, M.; Asakawa, J. I.; Tanaka, O. *Tetrahedron Lett.* **1977**, 175. (b) Tori, K.; Seo, S.; Yoshimura, Y.; Arita, H.; Tomita, Y. *Tetrahedron Lett.* **1977**, 179.

(8) Liu, H. W.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5591.

Chart I



(i) The difference in $\Delta\epsilon$ values of the two extrema of split CD curves, or "*A* values", of *dibenzoates* may be regarded as constants which are solely dependent on spatial arrangements of benzoate groups on the pyranose ring, and to the extent that the conformation remains unaltered, the values are not affected by nonchromophoric substituents such as –OAc, –OMe, –O–Si-*t*-BuMe_2, –Me. Thus the following *A* values were obtained (the signs are determined by the chirality) for the reference di-*p*-bromobenzoates: 1,2-ee, 62; 1,2-ea, 62; 1,2-aa, 6; 1,3-ee, 0; 1,3-ea, 16; *A* values were also found for seven other dibenzoates involving the 6-benzoate.

(ii) The *A* values of a tribenzoate can be approximated by the sum of the three-component dibenzoate moiety. For example, the *A* value, +137, of methyl α-D-galactopyranoside-2e,3e,4a-tri-*p*-bromobenzoate, 253 nm ($\Delta\epsilon$, +95)/236 nm ($\Delta\epsilon$, −42), is equal to the sum of the three units, +62(2e,3e) + 62(3e,4a) + 16(2e,4a) = +140. The additivity approximation is still valid for tetrabenzoates consisting of six interacting dibenzoate units.

The present micromethod consists of submitting the oligosaccharide to permethylation, methanolysis, and benzoylation; the nonanomeric hydroxyl groups involved in glycosidic linkages are thus converted into *p*-bromobenzoyloxy groups. The *A* values of the di- and tribenzoates derived from the branched pyranose group(s) then establish the spatial arrangement of benzoate groups and hence those of the hydroxyl groups which were involved in the branching, *independent of the pyranose species and without reference to authentic samples*.

The method is exemplified by application to two new potent molluscicidal saponins, balanitin-1 (Ba-1) (**1**) and balanitin-2 (Ba-2) (**5**).[9]

The aqueous methanol extract of *Balanites aegyptiaca*, a popular East African medicinal tree,[10] exhibited insect antifeedant (Mexican bean beetle), antimicrobial (*B. subtilis*, *P. cruotosum*, and *S. cerevisae*), and molluscicidal activity. Fractionation of 1 g of the crude methanol extract by acetone extraction, LH-20 chromatography, and droplet counter-current chromatography,[11] as monitered by molluscicidal bioassay using *Biomphalaria glubratus*, a South American snail which is the host of schistosomes,[12] yielded 31 mg of Ba-1 (**1**), 10 mg of Ba-2 (**5**), and 15 mg of Ba-3 (**4**).

Ba-1 (**1**) (100 μg or 0.1 μmol) (Chart I) was permethylated with $CH_3I/Me_2SO/NaH$ (Hakomori method),[13] methanolyzed

(9) The structure of balanitin-1 could be determined by FD–MS and NMR spectroscopy; however, in the case of balanitin-2, the results from ^{13}C NMR data were not decisive: Liu, H. W.; Nakanishi, K. *Tetrahedron*, in press.

(10) (a) Watt, J. M.; Breyer-Brandwijk, M. G. "Medicinal and Poisonous Plants of Southern and Eastern Africa"; E.&S. Livingstone Ltd.: Edinburgh, **1962**; p 1064. (b) Kokwaro, J. O. "Medicinal Plants of East Africa"; East African Literature Bureau: Kampala, 1976; p 34.

(11) Hostettmann, K.; Hostettmann-Kaldas, M.; Nakanishi, K. *J. Chromatogr.* **1979**, *170*, 355 and references cited therein.

(12) Nakanishi, K.; Kubo, I. *Isr. J. Chem.* **1977**, *16*, 28.

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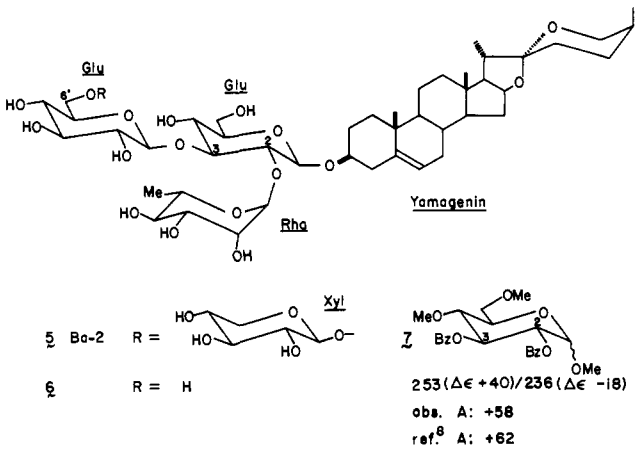
Chart II



by refluxing for 5 h in 8% HCl/MeOH, and *p*-bromobenzoylated after removal of solvent.[14] The two UV-absorbing products **2** (from Glu-2 unit in **1**) and **3** (from Glu-1 unit in **1**) were separated by TLC;[15,16] only the UV-absorbing products need be collected since all terminal units become permethylated methyl glycosides and therefore are "UV transparent".

CI-MS (CH_4 carrier gas)[17] of the two spots showed that they were a mono- and a dibenzoate. The amount of respective benzoates in the UV/CD cells were estimated from the standard UV ϵ values[18] at 244.5 nm without weighing of samples, and from this it was possible to measure the amplitudes of the CD curves.[17] For dibenzoate **3** the *A* value of the exciton-split CD curve at 253/238 nm was −6, which checks well with the reference value $A = 0$ for 1,3-ee dibenzoates.[8] This establishes that in balanitin-1, one of the glucose unit (Glu-1) is branched at C-2 and C-4 (1,3-ee).

Hydrolysis of Ba-2 (**5**) (5 mg) with $Ac_2O/AcOH/H_2SO_4$ at room temperature for 3 days cleaved the terminal xylose to give 1.2 mg of prosapogenin (**6**) (Chart II). Permethylation of **6**, followed by methanolysis with MeOH/HCl and *p*-bromobenzoylation, gave a single UV visible product which was separated by preparative TLC. CI-MS (CH_4) revealed the product to be a di-*p*-bromobenzoate. The amplitude of +58 of the split CD curve, as deduced from the UV absorbance, leads to a *vic*-ee dibenzoate arrangement, namely, **7**; the glucose is thus branched at C-2 and C-3 to the other sugars.[19]

Since only microgram quantities of material is employed and no reference sample is required, we believe that this method for determining the structure of branching points in oligosaccharides will be useful in structure elucidations of complex saccharides such as those encountered in serum glycoproteins and cell walls.[4] Micromethods for characterization of monosaccharides and determination of glycosidic linkages at nonbranching points in oligosaccharides are currently under development.[20]

(13) Hakomori, S. I. *J. Biochem.* **1964**, *55*, 205.
(14) Benzoylation was carried out by adding excess *p*-bromobenzoyl chloride to a solution of the residue in pyridine, keeping the mixture at 60 °C for 12 h, and quenching the reaction with methanol from a microsyringe. The solvent was evaporated after addition of a few drops of benzene (or toluene) to assist in removal of the pyridine.
(15) TLC was run on silica-precoated aluminum sheets (E. Merck, Darmstadt, G.F.R.), MeOH-$CHCl_3$ (2:98). The sensitivity of detecting the UV-absorbing products can be increased by using high-performance LC, μ-Porasil, MeOH-$CHCl_3$ (0.5:99.5).
(16) In the majority of cases, methanolysis of oligosaccharides affords one of the anomeric glycosides as the major product. In the present case, the benzoate **2** and 2,4-dibenzoate **3** only gave one anomeric glycoside as detectable spots. Since the CD data of α- and β-methylglycosides are similar, the anomeric configuration is immaterial for the argument; we have tentatively assumed it to be the α anomer since those are usually the major methanolysis products.
(17) A Finnigan 3300 instrument and a JASCO J-40 spectropolarimeter were employed.
(18) Standard ϵ values of *p*-bromobenzoates (in MeOH): mono, 19 500; di, 38 200; tri, 57 200; tetra, 76 400.[8]
(19) Although no attempts were made, the scale of the reaction can readily be reduced.
(20) Supported by NIH Grant CA 11572.

Amino Acids in the Hydrolysis Products of the Reaction of Carbon Vapor with Ammonia

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The carbon arc is a convenient source of both monoatomic and diatomic carbon which have been observed to undergo a wide variety of fascinating reactions.[1,2] We wish to report that co-condensation of arc generated carbon vapor with ammonia at −196 °C followed by hydrolysis produces amino acids in a reaction which has possible implications in the extraterrestrial synthesis of these compounds.

In a typical experiment, reactants were introduced under high vacuum and codeposited with arc generated carbon vapor on the walls of a reactor at −196 °C.[3] At the conclusion of the reaction, volatile components were removed under vacuum at room temperature and the residue hydrolyzed with 6 N HCl at 60 °C for 24 h. After removal of the solvent, the hydrolysate was heated with acidic *n*-butanol followed by trifluoroacetic anhydride in order to prepare the *N*-trifluoroacetyl *n*-butyl esters of the amino acids.[4] These derivatives were then analyzed by gas chromatography–mass spectrometry (GC–MS).[5] The presence of the primary amino acid products was also confirmed by using a Beckman amino acid analyzer. Reactants employed were (a) ammonia (b) ammonia and water, and (c) a mixture of NH_3, H_2O, and HCN. Table I lists the amino acids formed, along with their yields as determined by GC–MS, using these three sets of reactants. In all experiments, glycine, alanine, β-alanine, *N*-methylglycine, and aspartic acid were produced. When water was added to the reactants, serine was also generated. In each case, the mass spectrum of the amino acid derivative was identical with that of an authentic sample.

This unique reaction represents an example of the formation of amino acids in a system in which carbon vapor is the sole source of carbon.[6] Since arc generated carbon vapor is rich in C_1 and C_2,[1,2] it is likely that carbon enters into initial reactions in the form of one of these intermediates. With this fact in mind, we shall propose a tentative mechanism for the formation of glycine which is the major product.

The reaction of C_1 with NH_3 is expected to generate aminomethylene (**1**) which can either rearrange to methyleneimine (**2**) or lose H_2 to form HCN. Cacace and Wolf[7] have presented

$$C + NH_3 \rightarrow \underset{\mathbf{1}}{H-\ddot{C}-NH_2} \rightarrow HCN + H_2$$

$$\downarrow$$

$$\underset{\mathbf{2}}{H_2C{=}NH} \xrightarrow{HCN} \underset{\mathbf{3}}{NH_2-CH_2-CN} \xrightarrow{H_2O,\ H^+} \text{glycine}$$

evidence for the formation of **2** in the reaction of ^{11}C atoms with anhydrous NH_3. HCN, which was detected among the volatile products of the present reaction (Table 1), could subsequently add to **2** yielding aminoacetonitrile (**3**). The glycine would be generated from **3** in the hydrolytic workup. An examination of

(1) Skell, P. S.; Havel, J. J.; McGlinchey, M. J. *Acc. Chem. Res.* **1973**, *6*, 97-105.
(2) (a) Mackay, C. In "Carbenes"; Moss, R. A., Jones, M., Jr., Eds.; Wiley-Interscience: New York, 1975; Vol II, pp 1-42. (b) Shevlin, P. B. In "Reactive Intermediates"; Abramovitch, R. A., Ed.; Plenum Press: New York, 1980; Vol I, pp 1-36.
(3) The reactor is modeled after that described by Skell, P. S.; Wescott, L. D., Fr.; Golstein, J.-P.; Engel, R. R. *J. Am. Chem. Soc.* **1965**, *87*, 2829-2835.
(4) Roach, D.; Gehrke, C. W. *J. Chromatogr.* **1969**, *44*, 269-278.
(5) Leimer, K. R.; Rice, R. H.; Gehrke, C. W. *J. Chromatogr.* **1977**, *141*, 121-144.
(6) Harada and Suzuki (Harada, K.; Suzuki, S. *Nature (London)* **1977**, *266*, 275-276) have reported the formation of amino acids from elemental carbon by contact glow discharge electrolysis in aqueous ammonia.
(7) Cacace, F.; Wolf, A. P. *J. Am. Chem. Soc.* **1965**, *87*, 5301-5308.

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