and 0.07 g (7%) of the *E* isomer of **1a**.

The spectral data for **1a** follow: NMR ($CDCl_3$) δ 3.49 (t, 2, *J* = 7.2 Hz), 2.40 (t, 2, *J* = 7.2 Hz), 1.96–2.26 (m, 2), 1.76 (s, 3), 1.53–1.12 (m, 9), 1.04–0.70 (m, 3), 0.12 (s, 9); IR (neat) 3320, 2958, 2928, 2875, 2860, 1608, 1250, 1040 cm^{-1}; MS, *m/e* (relative intensity) 243 (1), 242 (3, M^+), 229 (2), 228 (8), 227 (17), 209 (5), 172 (7), 157 (5), 151 (3), 143 (2), 141 (2), 139 (2), 137 (2), 125 (4), 124 (4), 116 (4), 103 (6), 95 (4), 82 (49), 75 (77), 73 (100); mol wt calcd for $C_{14}H_{30}OSi$ 242.2064, found 242.2065. Anal. Calcd for $C_{14}H_{30}OSi$: C, 69.35; H, 12.47. Found: C, 69.50; H, 12.47.

The spectral data for the *E* isomer of **1a** follow: NMR ($CDCl_3$) δ 3.49 (t, 2, *J* = 7.2 Hz) 2.42 (t, 2, *J* = 7 Hz), 1.97–2.28 (m, 2), 1.82 (s, 3), 1.13–1.64 (m, 9), 0.75–1.04 (m, 3), 0.13 (s, 9); IR (neat) 3310, 2960, 2932, 2872, 2860, 1608, 1251, 1042; MS, *m/e* (relative intensity) 227 (20, M^+ – Me) 209 (3, M^+ – (Me + H_2O), 172 (4), 156 (5), 103 (12), 95 (11), 75 (75), 73 (100).

Preparation of 1b. A similar procedure on 10 times the scale with 1-(trimethylsilyl)-1-propyne (5.2 g, 46 mmol) gave, after chromatography on silica gel (4:1 pentane–ether), 4.04 g (52%) of **1b**: NMR (CCl_4) δ 3.32 (t, 2, *J* = 7.8 Hz), 2.8 (br s, 1, OH), 2.29 (t, 2, *J* = 7.8 Hz), 1.77 (s, 3), 1.70 (s, 3), 0.06 (s, 9); IR (neat) 3330 (br), 3000, 2950, 2900, 2875, 1610, 1450, 1380, 1370, 1260, 1250, 1040, 1015, 1000, 865, 830, 755, 680 cm^{-1}. Anal. Calcd for $C_9H_{20}OSi$: C, 62.72; H, 11.69; Si, 16.29. Found: C, 62.61; H, 11.89; Si, 16.07.

Preparation of 2a. *p*-Toluenesulfonyl chloride (0.4029 g, 2.1 mmol) was added to a soolution of **1a** (0.254 g, 1.05 mmol) in anhydrous pyridine. The solution was stirred 6 h at 25 °C and worked up to give 2.81 g (68%) of 95% pure **2a**: NMR (CCl_4) δ 7.74 (d, 2, *J* = 8 Hz), 7.33 (d, 2, *J* = 8 Hz), 3.81 (t, 2, *J* = 8 Hz), 2.47 (s, 3), 2.45 (t, 2, *J* = 8 Hz), 2.1 (br m, 2), 1.68 (s, 3), 1.3 (m, 8), 0.90 (br t, 3, *J* = 6 Hz), 0.07 (s, 9); IR (neat) 2960, 2935, 2860, 1600, 1460, 1375, 1250, 1188, 1176, 1097, 953, 833, 812, 755, 660 cm^{-1}.

Tosylate **2b** was prepared in a similar manner: NMR (CCl_4) δ 7.73 (d, 2, *J* = 8.0 Hz), 7.31 (d, 2, *J* = 8.0 Hz), 3.78 (t, 2, *J* = 8.0 Hz), 2.45 (s, 3), 2.43 (t, 2, *J* = 8.0 Hz), 1.80 (s, 3), 1.60 (s, 3), 0.07 (s, 9); IR (neat) 3070, 2957, 2930, 2895, 1607, 1600, 1450, 1375, 1360, 1250, 1187, 1175, 1095, 950, 855, 770 cm^{-1}.

Solvolysis of 2a. A solution of tosylate **2a** (220 mg, 0.55 mmol) in anhydrous *tert*-butyl alcohol (10 mL) was added to 22 mg of oil-free sodium hydride. The resulting solution was heated at reflux for 3 h and cooled to 25 °C. Water (20 mL) and hexane (30 mL) were added. The organic layer was washed four times with water and once with brine, dried ($MgSO_4$), and evaporated in vacuo to give 139 mg of product. Chromatography on silica gel (hexane) gave 69 mg (57%) of a 1.6:1 mixture of **4a** and **5a**. The NMR spectral data for **5a** were determined from this mixture: NMR (CCl_4) δ 4.75 (br s, 2), 2.0 (br m, 2), 1.3 (m, 8), 0.90 (t, 3, *J* = 6 Hz), 0.51 (s, 4), –0.08 (s, 9); ^{13}C NMR ($CDCl_3$) δ 154.0, 109.7, 36.3, 31.9, 29.4, 28.0, 22.7, 14.1, 10.5, 9.4, –2.6; GC/MS, *m/e* (relative intensity) 224 (0.3, M^+), 2.09 (0.1), 196 (0.1), 181 (0.2), 167 (0.1), 154 (1), 150 (1), 139 (1), 107 (2), 97 (1), 95 (1), 93 (1), 73 (100); GC t_R = 19.5 min (135 °C).

Isomerization of 5a to 4a. *p*-Toluenesulfonic acid (2 mg) was added to a solution of a 1.6:1 mixture of **4a** and **5a** (115 mg) in 5 mL of benzene. The resulting solution was refluxed for 24 h under nitrogen. Hexane and aqueous sodium bicarbonate were added. The organic layer was separated, washed with aqueous sodium bicarbonate, dried (Na_2SO_4) and evaporated to give 107 mg, which was purified by chromatography on silica gel (hexane) to give 45 mg (39%) of a 16:1 mixture of **4a** and **5a**. The spectral data of **4a** were determined from this mixture: NMR (CCl_4) δ 5.13 (br t, 1, *J* = 7 Hz), 1.95 (m, 2), 1.62 (br s, 3), 1.28 (m, 6), 0.89 (br t, 3, *J* = 6 Hz), 0.46 (s, 4), –0.09 (s, 9); in C_6D_6 the four-proton singlet at δ 0.46 was split into a multiplet at δ 0.38–0.56 typical of an AABB' system; ^{13}C NMR ($CDCl_3$) δ 138.4, 127.2, 31.5, 29.6, 28.0, 22.6, 17.5, 14.1, 10.6, 9.6, –2.7; IR (neat) 3075, 2935, 2860, 1460, 1378, 1260, 1250, 1190, 1180, 835, 748, 685 cm^{-1}; GC t_R = 17.4 min (135 °C); GC/MS, *m/e* (relative intensity) 224 (M^+, 0.5), 209 (0.2), 181 (0.2), 167 (0.1), 153(2), 109(1), 107(2), 97(2), 95 (2), 94 (3), 93 (5), 91 (1), 73(100). Anal. Calcd for $C_{14}H_{28}Si$: C, 74.91; H, 12.57. Found: C, 74.13; H, 11.89.

Solvolysis of 2b. Tosylate **2b** (0.653 g, 2.0 mmol) and anhydrous K_2CO_3 (0.57 g, 4.0 mmol) in anhydrous *tert*-butyl alcohol (40 mL) were heated at reflux under nitrogen for 14 h. A workup as above using pentane gave 0.287 g of product which was purified by evaporative distillation (25 °C, 0.5 torr) to give 86.3 mg of 90% pure **4b** (25%). The low yield is a result of the volatility of the desired product. A pure sample was obtained by preparative GC: NMR ($CDCl_3$) δ 4.76 (br, 1), 4.68 (br, 1), 1.74 (br s, 3), 0.54 (s, 4), –0.02 (s, 9); NMR (C_6D_6) δ 4.83 (br, 1), 4.80 (br, 1), 1.70 (br s, 3), 0.51–0.56 (m, 4), –0.04 (s, 9); ^{13}C NMR ($CDCl_3$) δ 149.9, 111.6, 23.3, 16.0, 9.7, –2.6; IR ($CDCl_3$) 3080, 3000, 2965, 2915, 2905, 1630, 1440, 1370, 1297, 1250, 1212, 1025, 835 cm^{-1}; GC t_R = 14.5 min (60 °C); MS, *m/e* (relative intensity) 154 (2, M^+), 139 (2), 112 (1), 111 (3), 99 (1), 97 (4), 86 (1), 85 (2), 83 (3), 81 (1), 79 (2), 77 (1), 73 (100).

Registry No. (*Z*)-**1a**, 83025-23-4; (*E*)-**1a**, 83025-24-5; **1b**, 83025-25-6; (*Z*)-**2a**, 83025-26-7; **2b**, 83043-20-3; (*E*)-**4a**, 83025-27-8; **4b**, 83025-28-9; **5a**, 83025-29-0; 1-(trimethylsilyl)-1-octyne, 15719-55-8; methyl bromide, 74-83-9; ethylene oxide, 75-21-8; 1-(trimethylsilyl)-1-propyne, 6224-91-5.

Circular Dichroic Method for Determining the Position of Glycosidic Linkages of Deoxy Sugar Moieties. Antitumor Antibiotic Chromomycin A_3

Raffaele Riccio[1] and Koji Nakanishi*

Department of Chemistry, Columbia University, New York, New York 10027

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We describe a three-step method for determining glycosidic linkages in complex natural products as exemplified by chromomycin A_3[2,3] (Chart I) which contains five 2,6-dideoxy sugars[4] attached to a tricyclic aglycon. The method can be carried out on a small scale and leads to determinations of intersugar linkages as well as configurational series, i.e., D or L series. We recently showed that an additivity relation exists in the amplitudes of exciton-split circular dichroism curves (CD) of pyranose benzoates,[5,6] and on this basis a submilligram method was developed to determine the position of glycosidic linkages at *branching points* in oligosaccharides without reference to authentic samples.[7] A variant of this method described below is applicable to various complex antibiotics, e.g., chromomycin A_3, which consists of sugar moieties linked to an aromatic aglycon; it is part of several micromethods[8] we are currently investigating to examine oligosaccharide structures.

The chromomycins,[2] olivomycins,[9] and mitramycins[10] belong to a group of structurally related antitumor anti-

(1) On leave of absence from Instituto per la Chimica di Molecole di Interesse Biologico, Arco Felice, Napoli, Italy.

(2) Miyamoto, M.; Kawamatsu, Y.; Kawashima, K.; Shinohara, M.; Tanaka, K.; Tatsuoka, S.; Nakanishi, K. *Tetrahedron* **1967**, *23*, 421.
(3) Harada, N.; Nakanishi, K.; Tatsuoka, S. *J. Am. Chem. Soc.* **1969**, *91*, 5896.
(4) Miyamoto, M.; Kawamatsu, Y.; Shinohara, M.; Nakadaira, Y.; Nakanishi, K. *Tetrahedron* **1966**, *22*, 2785.
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(9) Berlin, Y. A.; Esipov, S. E.; Kolosov, M. N. *Khim. Prir. Soedin.* **1967**, *5*, 567.
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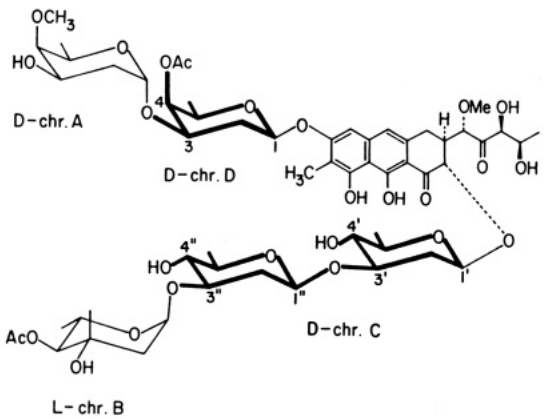
0022-3263/82/1947-4589$01.25/0

Chart I. Chromomycin A_3[a]



[a] The chromose C and D units of interest for the present glycosidic linkage studies are shown in thick lines.

biotics which are used clinically for cancer treatment. Recent extensive high-field proton and carbon-13 NMR studies[11] have led to a revision in one of the glycosidic linkages proposed by one of us;[1] namely, the linkage between the D-chromose A and D-chromose D units has been revised from α,1→4 (with 3_{eq}-OAc) to α,1→3 (with 4_{ax}-OAc, as depicted in Chart I). The structure of chromose D was originally determined as 2,6-dideoxy-3-*O*-acetyl-D-*lyxo*-hexopyranose[4] since this was the structure of the sugar originating from the chromose D moiety upon treatment of chromomycin A_3 with 50% AcOH at 65 °C for 30 h; the acetyl group had apparently migrated from C-4 to C-3 under these conditions. Because of the small number of hydroxyl groups in the chromoses, they provide a uniquely challenging situation in applying the current general method which is basically an extension of the dibenzoate chirality method.[12] For determination of the chirality between two benzoate groups, namely, the point of attachments and absolute configurations, both the anomeric hydroxyl as well as the hydroxyl involved in interglycosidic linkages had to be benzoylated and utilized in the present case. Attachment of an oligosaccharide(s) to a multifunctional aglycon moiety provides a situation which is shared by many important antibiotics. The current studies focus on the three inner saccharide units (depicted by thick lines in the structure), i.e., the glycosidic linkages of the D-chromose D unit and the two D-chromose C units.

Chromomycin A_3 was isolated by rotary locular counter-current chromatography[13] from a very crude mixture of the A_2, A_3, and A_4 compounds and various impurities. Attempts to permethylate the A_3 compound containing the air-sensitive aglycon by conventional Hakomori conditions[14] employing the methylsulfinyl anion resulted in poor yields due to destruction of the aglycon. The free hydroxyl groups were therefore permethylated by treating a solution of chromomycin A_3 in DMF/NaH with methyl iodide; the acetoxyl groups of chromose D (and chromose B) were hydrolyzed, presumably due to the fortuitous presence of water, and converted to methoxyl groups in this reaction. The fate of the labile chromomycinone moiety submitted to this permethylation reaction was not pursued, as it is

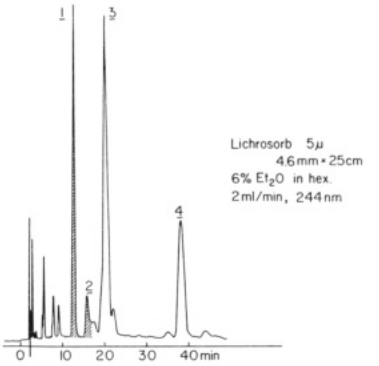
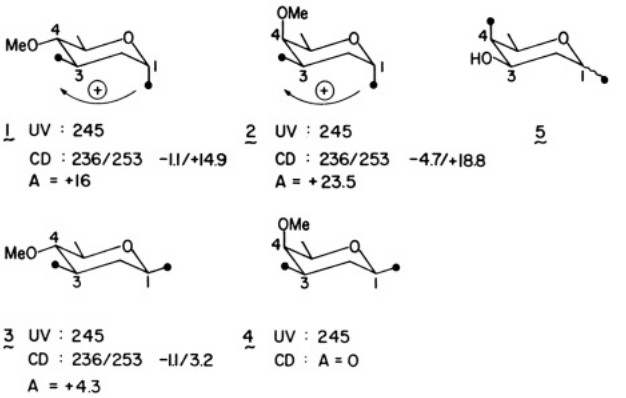


Figure 1. Analytical HPLC trace of *p*-bromobenzoate mixture. Peaks 1–4 correspond to structures 1–4. The shaded peaks 1 and 2 represent the two α-anomeric benzoates.

Chart II[a]



[a] The (*p*-bromobenzoyl)oxy groups are denoted by black circles. The UV and CD data were measured in MeOH. The CD data are the wavelengths (in nanometers) and ϵ values of the two split Cotton effects: *A* denotes the amplitudes of the split CD curves. The rounded arrows show the chiralities between the two benzoate groups in **1** and **2** (both positive).

not pertinent to the present studies. The methylated mixture was separated by preparative TLC, and the major product was hydrolyzed by being refluxed in 50% aqueous acetic acid for 7 h. After evaporation of solvent, the residue was per-*p*-bromobenzoylated, and the reaction mixture was separated by HPLC to afford the trace shown in Figure 1. Bands 1–4 were collected and resubmitted individually to HPLC to yield four pure samples of **1**–**4** (Chart II). ^1H NMR of these dibenzoates (see Experimental Section for data) showed that **1** and **3** were the two anomeric benzoates derived from chromose C and that **2** and **4** were derived from chromose D. As expected, the ratio of integrated areas of HPLC peaks 1 plus 3 (from chromose C) vs. those of 2 plus 4 (from chromose D) was approximately 2:1.

The CD amplitudes[15] accompanying structures **1**–**3** (Chart II) were derived in the following manner without weighing of the sample. Previous UV measurements of various hexopyranose *p*-bromobenzoates led to the following standard ϵ values: monobenzoate, 21 300; dibenzoate, 38 200; tribenzoate, 57 200; tetrabenzoate, 76 400.[5] In the present case, the mass spectral data showed that **1**–**4** were dibenzoates. The dibenzoate ϵ value of 38 200 was therefore used for estimating the concentrations of solutions submitted to UV and CD measurements; more-

(11) Thiem, J.; Meyer, B. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1331. Some of the chiral centers of the aglycon were erroneously represented and should be as described in ref 3 (private correspondence between Drs. J. Thiem and Y. Kawamatsu).

(12) Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989.

(13) Snyder, J., unpublished. A prototype instrument made by Tokyo Rika Kakai was employed. The chromomycin sample was a gift from Takeda Pharmaceutical Industries.

(14) Hakomori, S. I. *J. Biochem.* **1964**, *55*, 205.

(15) The amplitudes of exciton-split CD curves are defined as the difference in $\Delta\epsilon$ of the split extrema; the sign is defined as positive when the first Cotton effect (longer wavelength) is positive and vice versa: see ref 5 and 6.

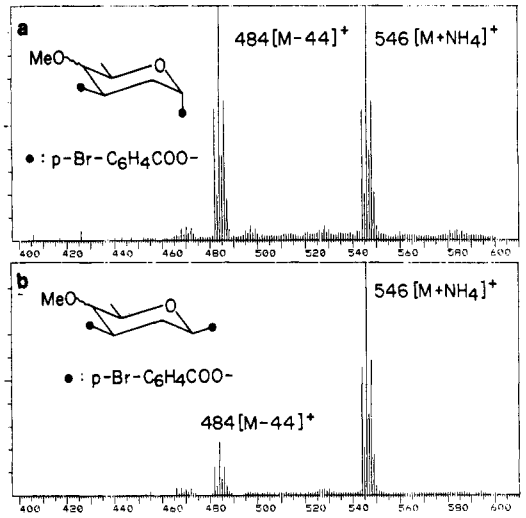


Figure 2. Desorption/chemical ionization mass spectral (D/CI-MS) with NH_3 as the reactant gas: (a) α-anomeric benzoates **1** and **2** (very similar), (b) β-anomeric benzoates **3** and **4** (very similar).

over, the large amplitudes of the exciton-split CD curves enable one to deal with only microgram quantities of sample.

Both ^1H NMR data (see Experimental Section) and CD data unambiguously showed that the chromose C and D units were involved in 1,3-linkages. Thus the large positivly split CD curves of the two α-anomeric benzoates derived from chromose C **1** (amplitude +16)[15] and chromose D **2** (amplitude +23.5) indicate that the dibenzoate units constitute a positive chirality and hence are located 1,3 on the sugars; namely, it is the 3-hydroxyl functions which are involved in interglycosidic linkages.[16] If the 4-hydroxyl functions were inolved in the linkages, the two benzoate groups would be 1,4 (see **5**), and no split CD curve would have been observed. The fact that the 1,3-diequatorial di-*p*-bromobenzoate **3** also exhibits a small but nevertheless split CD could be due to the distortion of the pyranose ring, restricted rotation of the benzoate group, and other factors, a phenomenon which has been encountered in other 1,3-diequatorial sugar dibenzoates.[5b]

The absolute configurations of the sugars were previously[4] based on the NMR correlation of C-1/C-5 relative configurations and application of the empirical Hudson isorotation rule.[17] The positive chiralities of the two dibenzoates **1** and **2** now establish that chromose C and D both belong to the D series.

It is interesting to note that (Figure 2) as measured by the mass spectral technique of desorption/chemical ionization with ammonia as the reactant gas,[18] there was a conspicuous difference in the M − 44 peaks between the α- and β-anomeric *p*-bromobenzoates. Thus besides the prominent $[M + NH_4]^+$ peaks at m/z 546, the two β-anomeric benzoates **1** and **2** showed intense m/z 484 peaks ($[M - 44]^+$), whereas the two α-anomeric benzoates **3** and **4** showed only weak peaks.

(16) The microgram-scale benzoylation of sugars with benzoyl chloride and pyridine usually yield the α(axial)-anomeric benzoate as the exclusive product. The fact that β(equatorial)-anomeric benzoates were the major products in the present case may be due to the fact that the chromoses are 2-deoxy sugars. See the following for HPLC separation of perbenzoates of some common sugars: Liu, H. W.; Nakanishi, K. *Tetrahedron* **1982**, *38*, 513.

(17) Hudson, C. S. *J. Am. Chem. Soc.* **1909**, *31*, 66; **1938**, *60*, 1537.

(18) For recent usages of this mass spectrum see: Hostettmann, K.; Doumas, J.; Hardy, M. *Helv. Chim. Acta* **1981**, *64*, 297. Lin, Y. Y.; Risk, M.; Ray, S. M.; Van Engen, D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773.

Experimental Section

The following instruments were used: NMR, Bruker WM-250; UV, JASCO UVIDEC-505; CD, JASCO-J40; mass spectroscopy, Ribermag R-10-10; the rotary locular countercurrent chromatograph was provided by Tokyo Rika Kikai; HPLC, Waters Model 6000A pump equipped with a U6K injector and a Schoeffel Model SF770 variable-wavelength detector. The ^1H NMR was run in $CDCl_3$; the chemical shifts are reported in parts per million on the δ scale relative to a Me_4Si internal standard; J values are reported in hertz.

Methylation of Chromomycin A_3. Chromomycin A_3 (25 mg) in 2 mL of DMF was slowly added under nitrogen to a stirred mixture of NaH (50 mg) in dry DMF (1 mL) cooled in an ice bath. The mixture was stirred for 10 min, and then CH_3I (1 mL) was added dropwise. The reaction mixture was kept for a further 10 min in the ice bath and then for 3 h at room temperature. The excess of NaH was destroyed by a few drops of methanol, and after addition of water, the mixture was extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), and evaporated under vacuum. The residue was loaded on a silica gel preparative plate (Whatman PLK5F, 1000 μm) and developed with chloroform–acetone (85:15). The fluorescent light-yellow band at R_f 0.6 was eluted to give a residue of 16 mg.

Hydrolysis with Acetic Acid. The above-mentioned "permethylated" chromomycin A_3 (16 mg) was hydrolyzed by being refluxed in 50% aqueous acetic acid (4 mL) for 7 h. The solution was then evaporated to dryness under reduced pressure to give a residue which was *p*-bromobenzoylated.

***p*-Bromobenzoylation and Separation of Benzoates by HPLC (Figure 1).** A solution of the mentioned residue in 2 mL of dry pyridine was treated with 60 mg of *p*-bromobenzoyl chloride, the mixture was stirred overnight at 60 °C under nitrogen, and chilled water was added to the solution, which after 20 min was extracted with chloroform. The organic layer was washed three times each with 0.1 N NaOH and water, the solvent was evaporated under vacuum, and the residual pyridine was removed by coevaporation with benzene.

The dark brown polar materials were removed by dissolving the residue in chloroform and passing it through a Pasteur pipet filled with a slurry of silica gel in chloroform. The front light-yellow portion was eluted with chloroform, and the eluate was evaporated to yield a residue (8 mg) containing the benzoates. The benzoate mixture was separated by HPLC under the following conditions: preparative HPLC Whatman Partisil M9 10/25, 5% ethyl ether in hexane, flow rate 5 mL/min, 260-nm detection. Figure 1 shows the result of an analytical HPLC of the benzoate mixture which was obtained under the conditions described in the figure.

1α,3-Di-*O*-(*p*-bromobenzoyl)-4-*O*-methyl-D-chromose C (1): ^1H NMR δ 6.51 (dd, J = 1, 4, H-1), 2.51 (ddd, J = 1, 5, 12, H-2e), 2.02 (ddd, J = 4, 11, 12, H-2a), 5.52 (ddd, J = 5, 9, 11, H-3), 3.15 (t, J = 9, H-4), 3.93 (dq, J = 6, 9, H-5), 1.35 (d, J = 6, Me), 3.53 (s, OMe), 7.92 (2 H), 7.30 (2 H), and 7.60 (4 H) (all d's, J = 9, aromatic H's); D/CI-MS (NH_3) m/z 526, 528, 530 ($[M + NH_4]^+$), 482, 484, 486 ($[M - 44]^+$).

1α,3-Di-*O*-(*p*-bromobenzoyl)-4-*O*-methyl-D-chromose D (2): ^1H NMR δ 6.53 (br d, J = 3, H-1), 2.62 (br dd, J = 5, 12, H-2e, broadened by W coupling to H-4), 2.53 (dt, J = 3, 12, H-2a), 5.54 (ddd, J = 3, 5, 12, H-3), 3.58 (br, $W_{1/2}$ = 4 Hz, H-4), 4.18 (br q, J = 6, H-5), 1.29 (d, J = 6, CH_3), 3.55 (s, OCH_3), 7.92 (2 H), 7.90 (2 H), 7.60 (2 H), 7.59 (2 H) (all d's, J = 9, aromatic H's); D/CI-MS (NH_3) m/z 526, 528, 530 ($[M + NH_4]^+$), 482, 484, 486 ($[M - 44]^+$).

1β,3-Di-*O*-(*p*-bromobenzoyl)-4-*O*-methyl-D-chromose C (3): ^1H NMR δ 6.02 (dd, J = 2, 10, H-1), 2.57 (ddd, J = 2, 5, 12, H-2e), 1.97 (dt, J = 10, 12, H-2a), 5.22 (ddd, J = 5, 9, 12, H-3), 3.13 (t, J = 9, H-4), 3.62 (dq, J = 6, 9, H-5), 1.40 (d, J = 6, Me), 3.50 (s, OMe), 7.90 (2 H), 7.89 (2 H), 7.59 (2 H), and 7.55 (2 H) (all d's, J = 9, aromatic H's); D/CI-MS (NH_3): m/z 526, 528, 530 ($[M + NH_4]^+$), 482, 484, 486 ($[M - 44]^+$).

1β,3-Di-*O*-(*p*-bromobenzoyl)-4-*O*-methyl-D-chromose D (4): ^1H NMR δ 5.99 (dd, J = 2, 10, H-1), 2.19 (ddd, J = 2, 5, 12, H-2e, broadened by W couplings to H-4), 2.38 (dt, J = 10, 12, H-2a), 5.23 (ddd, J = 3, 5, 12, H-3), 3.46 (br d, J = 3, $W_{1/2}$ = 6 Hz, H-4), 3.80 (dq, J = 1, 6, H-5), 1.36 (d, J = 6, Me), 3.56 (s, OMe),

7.93 (4 H), 7.60 (2 H), and 7.56 (2 H) (all d's, $J = 9$, aromatic H's); D/CI-MS (NH_3) m/z 526, 528, 530 ($[M + NH_4]^+$), 482, 484, 486 ($[M - 44]^+$).

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Vacuum Dry Column Chromatography

Eric Jan Leopold

Chemistry Department, Stanford University, Stanford, California 94305

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Today's organic chemist is faced with a dry column of techniques for purifying mixtures on a large or small scale. Unfortunately, the recent advances in separation science often involve expensive equipment.

Reported here is the development of vacuum dry column chromatography (VDCC). This method involves no expensive or exotic equipment. VDCC has been used successfully in our laboratories in separating large and small samples with a significant saving of time. The use of dry-column chromatography (DCC) was first detailed by Loev and Snader.[1]

In the process of dry-column chromatography, a nylon tube is sealed at one end and packed with dry adsorbent. The compound to be purified is placed at the top of the column and a small puncture hole is made at the column base. The eluting solvent is added at the top of the column and is allowed to flow by gravity to the bottom. The various impurities are separated from the desired compound(s) in the fully developed column. Although elution for a small column by using DCC can be carried out in 30 min, with larger columns much longer elution times are required.

The technique described here makes use of a vacuum during the process of dry-column chromatography. Thus, the vacuum is applied to the base of the dry column and the eluting solvent is pulled by the vaccum down the column, the advantage being that the time for elution is greatly reduced. The rapid elution has no effect on the resolving power of the adsorbent.

Another advantage of VDCC is that compounds that are unstable to silica gel or other adsorbents are in contact with the adsorbent for a minimum length of time. Further, if an inert gas is used during the preparation of the column as described below, the elution can be carried out in the absence of air in the case of air-sensitive compounds. In this method, 160-gauge nylon tubing, of various circumferences (2, 3, 4, 5, or 6 in.)[2] is used to contain the adsorbent such as silica gel or alumina, but in no way is the process limited to these two substances.

For mixtures of average separation, VDCC has been used successfully to purify 22 g of material in a 28 × 2 in. (circumference) nylon column by using 700 g of silica gel. The impurities were located at TLC R_f values of 0.25 and 0.7 while the desired material had an R_f of 0.4. On a smaller scale, 120 mg of an impure compound was chromatographed by VDCC on 50 g of silica gel in a 20 × 2 in. (circumference) nylon column. In this case, the desired compound had a TLC R_f of 0.4 while the impurities had values R_f of 0.04 and 0.5. For difficult separations, see the Experimental Section.

The use of vacuum[3] and pressure[4,5] in chromatography processes using glass columns to contain the adsorbent has been described. In the vacuum process, a vacuum pump and coolent traps are required. In the pressure processes, a source of compressed air for medium and large columns is necessary. These processes are similar to conventional liquid chromatography in that fractions of eluant are collected and analyzed. With VDCC, fraction collection is unnecessary and a water aspirator vacuum is sufficient.

VDCC uses deactivated adsorbent, since such adsorbent has greater resolving power than undeactivated adsorbent.[1] Relative flow or R_f data from thin-layer chromatography (TLC) plates is transferable to a vacuum dry column. In this way, the approximate location of the desired compound(s) can be anticipated.

The VDCC process makes use of single solvent systems for elution. If the best separation calls for the use of a mixed solvent system, then it is necessary to pretreat the water-deactivated adsorbent with 10% v/w of solvent mixture (see Adsorbents).

In general, if the TLC R_f of a compound is greater than 0.6, then the compound elutes at a higher R_f on the column than on a TLC plate. Conversely, if the R_f of a compound is less than 0.25, then the compound elutes at a lower R_f on the column than on a TLC plate. When the R_f is in the range of 0.2–0.6, the TLC data and the position on the column are closely comparable. If a difficult separation is to be carried out, then more than a 50:1 ratio of adsorbent to substrate is used, and tubing with a high ratio of length to diameter is selected.

The original article by Loev[1] describes the procedure for location of separated compounds by scanning with short wavelength ultraviolet light. This method is limited to the use of ultraviolet light transparent solvents and to compounds capable of "blanking out" the fluorescence of indicators. The exact location of separated compounds in VDCC is determined by using a small syringe having a stainless-steel needle to puncture the nylon wall and remove 1–10-μL samples for analysis.[6]

Experimental Section

Adsorbents. In the VDCC method, the adsorbent is deactivated by the addition of 5–15% v/w of water and is allowed to stand for more than 12 h or is rotated in a flask on a rotary evaporator without the application of vacuum for 3 h. If a mixed solvent is to be used, the adsorbent is treated with a 10% v/w solvent mixture along with the water for deactivation. The treated adsorbent is allowed to stand for more than 12 h or is rotated in a flask by using a rotary evaporator without a vacuum for 3 h.[1]

Column Apparatus. The nylon tubing is attached at one end to the exterior of an appropriately sized Büchner funnel by means of rubber bands (adhesive tape may also be used). A clamp is used to make the junction secure. The Büchner funnel is modified and has a three-way vacuum stopcock on the stem (Figure 1). Other methods of attaching the nylon tubing may be used, including attaching the nylon tubing to an appropriately sized glass bushing adapter and securing it with rubber bands, tape, and/or

(1) Loev, B.; Snader, K. M. *Chem. Ind. (London)* **1965**, 15. See also: Loev, B.; Goodman, M. *Ibid.* **1967**, 2026. Perry, A.; Oss, A. Eds. "Progess in Separation and Purification"; Interscience: New York, 1970; Vol. III, p 73.

(2) Nylon tubing is available as Copol 8(YK) 160-gauge nylon tubing from Walter Coles and Co., Ltd., Plastics Works, 47/49 Tanner Street, London, SE 1, England.

(3) Targett, N. M.; Kilcoyne, J. P.; Green, B. *J. Org. Chem.* **1979**, *44*, 4962.

(4) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(5) Taber, D. F. *J. Org. Chem.* **1982**, *47*, 1351.

(6) Hadd, H. E.; Caspi, E. *J. Chromatogr.* **1972**, *71*, 353. These authors describe puncturing the nylon wall with glass pipettes. The use of a syringe is the suggestion of John Thomas Welch.

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