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Absolute Configuration and Enantioselective Synthesis of Spiculisporic Acid

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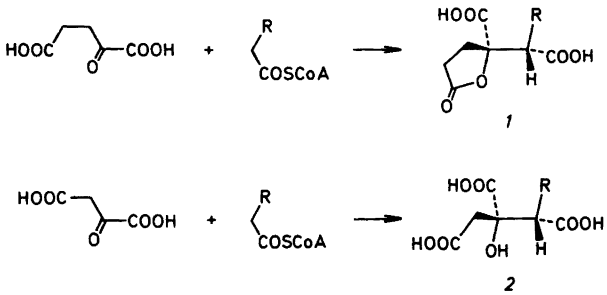
The absolute configuration of spiculisporic acid (*1*), obtained from *Penicillium spiculisporum* Lehman, has been determined as (3*S*,4*S*) by a degradation into (2*S*,3*S*)-decylcitric acid (*2*), a metabolite of a variant of this fungus. The key step in the enantioselective synthesis of *1* from D-glucose is the nucleophilic addition to the ketone *6*. Whereas the organolithium reagent *11* (a new homoenolate anion equivalent) leads mainly to the desired equatorial addition, the corresponding Grignard reagent leads to the opposite stereochemistry. There is evidence (GLC–MS) that *P. spiculisporum* also produces 3-hydroxy-1,3,4-dodecanetricarboxylic acid and 3-hydroxy-1,3,4-tridecanetricarboxylic acid.

Spiculisporic acid (*1*) is obtained by recrystallisation of the precipitate formed on acidifying the culture broth of *Penicillium spiculisporum* Lehman and other *P.* species.[1,2] It has been reported, however, that the actual metabolite is not the lactone *1* but the corresponding hydroxy tricarboxylic acid,[3] here called secospiculisporic acid. The biosynthesis involves a condensation of lauroyl-CoA with α-ketoglutarate[4] (Scheme 1); the condensing enzyme has been purified and characterized.[5] Gatenbeck and Måhlén found that cultures of the fungus gradually lost the ability to synthesize secospiculisporic acid and a variant strain appeared which instead synthesized a single stereoisomer of decylcitric acid (*2*).[6,7] Måhlén subsequently purified and characterized the enzyme which mediates the synthesis of *2* from lauroyl-CoA and oxaloacetate (Scheme 1).[8] It was suggested that the active site of the enzyme had changed and no longer accepted α-ketoglutarate as substrate but only the lower homologue oxaloacetate.[5] If this interpretation is correct, *1* and *2* would most probably have the same absolute configuration. The configuration of *2* has been determined[9] as 2*S*,3*S* thus indicating



Scheme 1. Biosynthetic precursors of spiculisporic acid (*1*) in *Penicillium spiculisporum* Lehman and of decylcitric acid (*2*) in a variant of the same fungus, (R=n-$C_{10}H_{21}$).

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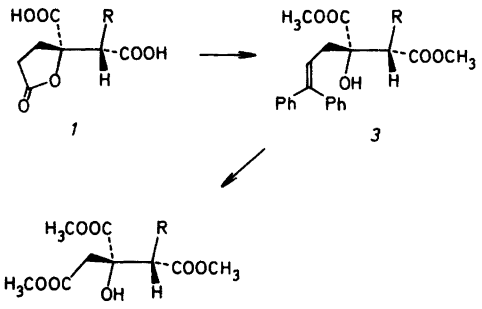
that the attack on oxaloacetate by a nucleophilic species derived from lauroyl-CoA occurs at the *si* face of oxaloacetate. In the present communication, the absolute configuration of *1* is reported.

We also describe an enantioselective synthesis of *1* in which the carbons C-2 and C-3 in D-glucose appear in the product *1* as its two asymmetric carbons. The use of carbohydrates as starting materials in the preparation of non-carbohydrate compounds has received considerable attention in recent years;[10–12] the present synthesis (Scheme 3) represents an extension of the strategy which we previously applied to the synthesis of fluoro- and methylcitric acids.[13]

ABSOLUTE CONFIGURATION OF *1*

Hydrolysis of the lactone ring of *1* yields a decylhomocitric acid. Removal of a methylene group from this acid gives one of the four characterized[9] stereoisomers of decylcitric acid. This may be achieved by the Barbier-Wieland degradation,[14] in which an ester of the carboxylic acid reacts with two equivalents of phenylmagnesium bromide. For our purposes, a selective reaction at the lactonic carbonyl group was required. However, experiments in which we used *1* or its dimethyl ester and phenylmagnesium bromide or phenyllithium in various amounts showed poor selectivity. A multi-component mixture obtained from *1* and three equivalents of phenyllithium was esterified with diazomethane and chromatographed on silica gel to afford the desired intermediate *3* (Scheme 2)



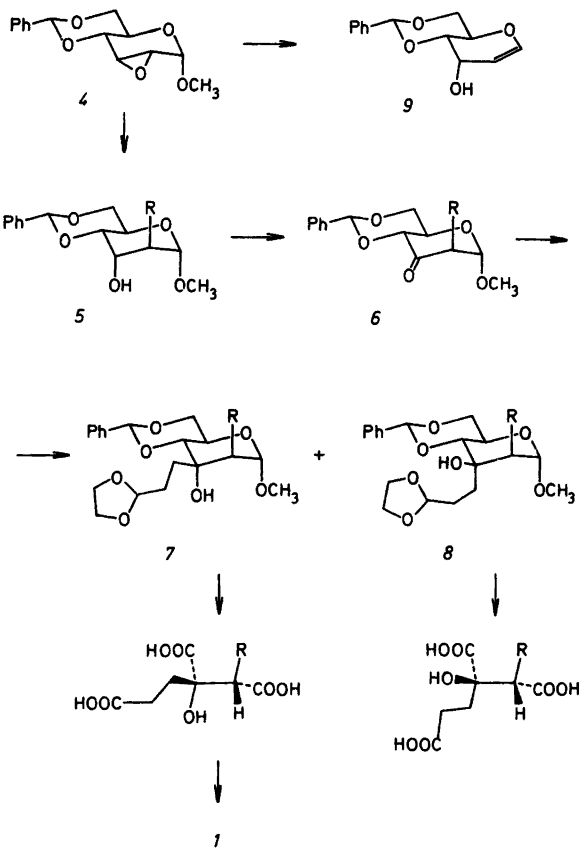
Scheme 2. A modified Barbier-Wieland degradation of spiculisporic acid into the trimethyl ester of *2* shows that the former is the γ-lactone of (3*S*,4*S*)-3-hydroxy-1,3,4-tridecanetricarboxylic acid (*1*, R=n-$C_{10}H_{21}$).

in a 13 % yield; the characteristic olefinic proton appeared as an NMR triplet at δ 5.98. Oxidative cleavage of the olefinic bond of *3* with ruthenium tetroxide and reaction with diazomethane gave trimethyl decylcitrate in a 48 % yield. Its ^{1}H NMR spectrum and optical rotation were indistinguishable from those of the trimethyl ester of *2* and thus it was clear that *1*, too, has the *S,S* configuration (Scheme 1). This result agrees with the suggestion[5] that the change in metabolite production, from *1* to decylcitric acid, is due to a minor modification of the active site of the enzyme involved.

ENANTIOSELECTIVE SYNTHESIS OF *1*

The synthetic pathway for *1* is based on our observation that some 2-deoxy-3-*C*-substituted sugars can be degraded into carboxylic acids possessing a tertiary α-hydroxy group by reaction with alkaline aqueous permanganate.[13] A retrosynthetic analysis shows that compound *7* is a suitable intermediate for a synthesis of *1*, incorporating this type of degradation. The reaction steps for the synthesis of *7* are outlined in Scheme 3.

Two methods for preparing 2-*C*-alkyl-2-deoxy-3-uloses have been described. The alkylation of a lithium enolate ultimately leads to compounds having an equatorial substituent[15] at C-2 and thus is inapplicable to the synthesis of *6*. The second method consists of a regioselective nucleophilic ring opening of a 2,3-epoxide, followed by oxidation of the secondary hydroxyl group to a keto group. Reaction between lithium dimethylcuprate and the readily available epoxide *4* gave methyl 4,6-*O*-benzylidene-2-deoxy-2-*C*-methyl-α-D-altropyranoside, the methyl analog of *5*, in about 70 % yield.[16,17] However, an attempt to prepare *5* by an analogous reaction between *4* and lithium didecylcuprate yielded the allylic alcohol *9* as the main product and only a low yield of *5*. Compound *9* has been identified in reactions between *4* and certain organometallic reagents.[18] Recently Lipshutz *et al.* have found that higher-order mixed organocuprates of the general formula $R_2Cu(CN)Li_2$ are superior to the Gilman reagents R_2CuLi for the ring-opening of epoxides.[19] Thus, treatment of *4* with 2.5 equivalents of the reagent formed from copper(I) cyanide and decyllithium in the molar ratio 1:2



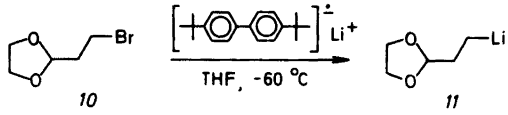
Scheme 3. Enantioselective synthesis of *1*. Reaction between ketone *6* and the organolithium reagent *11* yields an 8:1 mixture of *7* and *8*; reaction with the corresponding Grignard reagent yields a 1:6 mixture of the same compounds. R=n-$C_{10}H_{21}$.

gave *5* in an improved yield of 46 %; the main product was, however, still *9* (52 %). In the NMR spectra of *5* and its oxidation product *6*, the anomeric proton appeared as a singlet. After base-catalysed epimerisation of *6* at C-2 (*Cf.* Ref. 17), the anomeric proton showed a vicinal coupling of 3.9 Hz. Since the anomeric proton in some methyl 4.6-*O*-benzylidene-α-D-hexopyranosides shows a smaller coupling (0.6–1.7 Hz) to an equatorial H-2 than to an axial (3.3–3.8 Hz),[20] we may thus conclude that the decyl group is located at C-2 and, most probably, in the axial position. This is the expected mode of ring-opening in the reaction with *4*.[21]

The nucleophilic addition of a propionic acid moiety to the carbonyl group of *6* requires a homoenolate anion or its equivalent as reagent.[22] Reactions with the dianion of propionic acid[23] or *N*-phenylpropionamide,[24] however, met with only limited success and we therefore tried reagents of lower oxidation state, *e.g.* aldehyde acetals. The use of this latter functionality adds no extra steps to the overall synthesis, since the acetal is converted into a carboxylic group during the oxidative degradation of the carbohydrate moiety. The Grignard reagent derived from 2-(2-bromoethyl)-1,3-dioxolan (*10*)[25] has been used by several workers as a synthon for $^{\ominus}CH_2CH_2CHO$.[26] It reacted somewhat sluggishly in THF with the ketone *6*; the reaction required 80 min at 0 °C to attain completion. Two stereoisomeric addition products, formed in the approximate ratio 1:6, were characterized by ^{13}C and ^{1}H NMR. The major isomer was

subjected to oxidative degradation. Cleavage of the three acetal functions by acidic hydrolysis, subsequent treatment with potassium permanganate in alkaline solution, acidification and esterification with diazomethane, and finally purification by HPLC gave a trimethyl ester which proved to be a diastereomer (3*R*,4*S*) of the trimethyl ester prepared from *1* (^{1}H NMR, HPLC retention time). The preponderant stereoisomer was thus *8*. It was therefore desirable to find an experimental modification which would give *7* as the major isomer.

Previous attempts to prepare the organolithium reagent *11* from *10* and lithium metal have been unsatisfactory. Eaton *et al.* concluded that *11* reacted further as it was formed.[27] We found, however, that *11* could be generated



10 *11*

smoothly by the new technique for the preparation of organolithium compounds described by Freeman and Hutchinson.[28] When *10* was allowed to react at *ca.* −60 °C with the radical anion obtained from lithium and 4,4'-di-*tert*-butylbiphenyl in THF, *11* was rapidly produced. The addition reaction with *6* was complete within 15 min at *ca.* −60 °C and thus the new synthon *11* is much more reactive than the corresponding Grignard reagent. HPLC analysis showed that *7* and *8* had been formed in an approximate ratio of 8:1. On flash chromatography on silica gel, *7* was obtained in a pure form with a combined yield of *7* and *8* of 82 %. Oxidative degradation of *7* as described for the preparation of *e.g.* methylcitric acids[13] gave, however, only a 10 % yield of a tricarboxylic acid (the yields of the citric acids were about 30 %). To minimize side reactions, a milder hydrolysis was carried out which cleaved only the benzylidene and dioxolan acetal functions. Reduction with sodium borohydride, hydrolysis of the remaining acetal at C-1, oxidation with alkaline aqueous permanganate, acidification, reaction with diazomethane, and purification on silica gel gave a somewhat higher yield of degradation product (15 %) than before. The trimethyl ester formed was indistinguishable (^{1}H NMR, HPLC), from the trimethyl ester obtained from *1* by lactone scission and reaction with diazomethane. Further, alkaline hydrolysis of the trimethyl ester obtained from *7* followed by acidification gave a carboxylic acid which was indistinguishable from spiculisporic acid (m.p., optical rotation).

CONCLUDING REMARKS

NMR evidence indicates that *6* exists preponderantly in the chair form. The anomeric proton is seen as a singlet ($CDCl_3$) and this fact indicates only a minor population of conformations in which the pyranose ring is in a boat or skew boat form. Molecular models show that, in these forms, the dihedral angle between H-1 and H-2 is close to 180° and the coupling constant is therefore expected to be about 10 Hz. Although the chair form of *6* seems to preponderate, any minor conformer can play a major role in determining the stereochemistry in the reactions of *6* with the organometallic reagents provided that its relative reactivity is high enough.[29] Also differences between the organometallic reagents, may be decisive. Organolithium reagents are more reactive than Grignard reagents and their additions to carbonyl groups are less sensitive to steric hindrance.[30] The formation of chelates involving the carbonyl oxygen and another oxygen atom in the same molecule may govern the stereochemistry of an addition to the carbonyl group; organomagnesium reagents are regarded as better chelators than organolithium reagents.[31] In view of the above factors, it seems probable that *11* mainly attacked the equatorial (β) side of the chair form of *6*. The corresponding Grignard reagent must have reacted preferentially on the α side, attacking the chair or any minor form of *6*. The reactions of methyllithium and methylmagnesium halide with carbohydrate ketones show widely different stereochemical courses.[32,33]

Acidification of the culture broth of *Penicillium spiculisporum* Lehman, extraction with chloroform-ethanol, treatment of the extracted material to induce lactonisation, and finally reaction with diazomethane and analysis by capillary column GLC–MS showed a major peak from the dimethyl ester of *1* and some minor peaks at shorter retention times. The mass spectra indicate that the minor peaks correspond to the dimethyl esters of those lower homologues of *1*, in which the decyl group is replaced by an

octyl and a nonyl group respectively. These two minor compounds have not been detected before; they amount to *ca.* 5 % and <1 % of *1*, respectively. Trimethyl decylcitrate was not detected. In studies of the enzyme which mediates the condensation of lauroyl-CoA with α-ketoglutarate to secospiculisporic acid, it was found that replacement of lauroyl-CoA with decanoyl-CoA led to a decrease in relative reaction rate from 100 to 20–25 %.[5] It is thus possible that secospiculisporic acid and its minor homologues are formed by the same enzyme.

EXPERIMENTAL

General methods have been described elsewhere.[13] Analytical and preparative HPLC was performed on a silica gel column (LiChrosorb Si60, 5 μm, 4×250 mm, Merck), eluting with 2,2,4-trimethylpentane–ethyl acetate (solvent system A–B), unless otherwise stated. The relative amounts of *7* and *8* were determined with a differential refractometer (R 403, Waters) coupled to an electronic integrator (Hewlett-Packard 3390 A). Flash chromatography[34] of gram quantities was carried out, using a column of Kieselgel 60 (Merck. 230–400 mesh, 6×15 cm); a smaller column was used for sub-gram quantities. TLC was run on silica gel 60 F_{254} (Merck). NMR spectra were recorded on a JEOL JNM–FX 100 instrument and the UV spectrum on a Beckman DK 2 spectrometer. GLC–MS was performed using an SE-54 capillary column (25 m, splitless mode) and helium as carrier gas; temperature program: 100/8/280 °C. The column was linked to a Kratos MS 50 spectrometer (ionisation voltage, 70 eV) and the data program DS 55S was used for the processing.

Investigation of natural products. The acidified (pH 1–2) culture broth (1.2 l) was extracted with chloroform-ethanol (3:2, 5×250 ml). The organic layers were combined and dried (Na_2SO_4) and an aliquot was taken out and was freed from solvents. Secospiculisporic acid and its homologues were converted into lactones by storage over P_2O_5 at *ca.* 1 Pa (5 d, *ca.* 60 °C). After reaction with ethereal diazomethane, the mixture was investigated by GLC–MS.

Octyl homologue of dimethyl ester of 1: *m/z* =328 lacking. 296 (3 %), 269 (82), 241 (67), 237 (8), 209 (22), 185 (9), 181 (21), 157 (15), 115 (41), 55 (100), 43 (17), 41 (23).

Nonyl homologue of dimethyl ester of 1: *m/z*=342 lacking, 310 (3), 292 (4), 283 (71), 255 (58), 251 (8), 223 (24), 195 (20), 166 (14), 157 (17), 115 (42), 55 (100), 43 (28), 41 (26).

Dimethyl ester of 1: *m/z*=356 lacking, 324 (4), 297 (73), 269 (54), 265 (8), 237 (18), 209 (17), 157 (14), 115 (36), 55 (100), 43 (24), 41 (22).

Reaction of 1 *with phenyllithium.* An ethereal solution of phenyllithium (0.57 M, 3.2 ml, 1.8 mmol) was added dropwise (3 min, N_2 atmosphere) to a stirred and cooled (*ca.* −60 °C) solution of *1* (201 mg, 0.61 mmol) in THF (25 ml). When the addition was complete, the mixture was allowed to reach 23 °C (*ca.* 1 h) and, after 18 h, hydrochloric acid was added (1 M, 10 ml). The organic layer was separated, dried (Na_2SO_4), and treated with excess ethereal diazomethane. After filtration and evaporation of the solvents (*ca.* 100 Pa, 16 h), a residue (280 mg) was obtained. TLC (silica gel, methylene chloride–ether, 20:1) showed strongly UV-absorbing spots at R_F 0.97, 0.64, 0.56, 0.26, and 0.07; the dimethyl ester of *1* was present (H_2SO_4) at R_F 0.46. The component giving the most intense UV response (R_F 0.56) was isolated by chromatography on silica gel (50 g, 230–400 mesh) using methylene chloride–ether (20:1) as eluant. The colourless oil obtained was identified as *3*; yield of chromatographically pure material: 40 mg (13 %). ^{1}H NMR ($CDCl_3$); δ 7.5–7.0 (m, 10H), 5.98 (t, 1H, *J* 7 Hz), 3.71 (s, 3H), 3.66 (s, 1H), 3.62 (s, 3H), 3.0–2.4 (m, 3H), 2.0–1.0 (m, 18H), 0.85 (t, 3H); UV (EtOH): λ_{max} 251 nm (log ε, 4.2); lit. values for other 1,1-diphenyl-1-alkenes: 251 (4.08);[35] 249 (4.33) and 255 (4.36).[36]

Oxidative degradation of 3 was performed using the technique of Stork *et al.*[36] Sodium periodate (106 mg) was added in portions over a period of 24 h (23 °C) to a stirred mixture of *3* (18 mg), ruthenium dioxide (2.9 mg), acetone (3 ml), and water (1 ml). Isopropanol (*ca.* 1 ml) was added to reduce RuO_4 and then 0.1 M hydrochloric acid (10 ml). Extraction with methylene chloride (2×10 ml), drying (Na_2SO_4), filtration, concentration, treatment with excess ethereal diazomethane, and concentration yielded a crude product (16 mg). Purification by preparative HPLC (methylene chloride–ether, 20:1) gave trimethyl decylcitrate (6.5 mg, 48 %); ^{1}H NMR ($CDCl_3$): indistinguishable from that of trimethyl (2*S*,3*S*)-decylcitrate[9]; $[\alpha]_D^{24}$ −7.9°; $[\alpha]_{546}^{24}$ −11.8° (*c* 0.5, chloroform); authentic trimethyl (2*S*,3*S*)-decylcitrate[9] showed $[\alpha]_D^{24}$ −9.3°; $[\alpha]_{546}^{24}$ −12.8° (*c* 0.6, chloroform).

Methyl 4,6-O-benzylidene-2-C-decyl-2-deoxy-α-D-altropyranoside (5). An ethereal solution of decyllithium (1.46 M) in diethyl ether was prepared from decyl chloride and lithium containing 2 % sodium (Lithium Corp. of America, Inc.). A 164 ml aliquot of this solution (240 mmol) was added under stirring (N_2 atmosphere) to a cooled (−78 °C) suspension of copper(I) cyanide (11.2

g, 125 mmol) in tetrahydrofuran (THF, 200 ml). The mixture was warmed to 0 °C and kept at this temperature for 5 min to give a clear solution of the cuprate reagent. Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside[37] (*4*, 13.2 g, 50 mmol) was then added in portions in solid form at 0 to −5 °C and after 1 h at this temperature, the reaction mixture was poured onto ice-aqueous ammonium chloride solution (300 ml). Extraction with ether (3×100 ml), drying ($MgSO_4$), and evaporation of the solvent gave a crude reaction product. This was subjected to flash chromatography using toluene–ethyl acetate (8:1) as eluant. The desired product *5* (9.2 g, 46 % yield) was eluted before *9* (6.0 g, 52 %). Compound *5* (chromatographically pure) melted at 30–35 °C but was not recrystallised. ^{1}H NMR ($CDCl_3$): δ 7.6–7.3 (m, 5 H), 5.62 (s, 1 H), 4.56 (s, 1 H), 4.4–3.6 (m, 5 H), 3.41 (s, 3 H), 3.91 (d, 1 H, *J* 7.3 Hz), 2.1 (m, 1 H), 1.5–1.1 (m, 18 H), 0.88 (t, 3 H). ^{13}C NMR ($CDCl_3$): 137.9, 129.0, 128.2, 126.6, 102.3 (two peaks), 77.2, 69.5, 69.2, 58.7, 55.4, 45.9, 32.1, 30.2, 29.8, 29.7, 29.6, 28.3, 22.9, and 14.4 ppm; $[\alpha]_D^{20}$ +46° (*c* 1.0, CH_2Cl_2).

Methyl 4,6-O-benzylidene-2-C-decyl-2-deoxy-α-D-arabino-hexo-pyranosid-3-ulose (6) was prepared by oxidation of *5* (4.5 g, 11.1 mmol), using the technique described by Garegg and Samuelsson[38] but with eight equivalents of chromium(VI) oxide (8.9 g) and corresponding excesses of other reagents. After a reaction time of about 5 min, ethyl acetate (250 ml) was added. The solution was decanted and then passed through a silica gel column (see above), using ethyl acetate. Evaporation of the solvent, and codistillation with toluene (3×30 ml, 30 °C) gave *6* as a colourless oil (3.9 g, 87 %) which subsequently crystallised. Recrystallization from ethyl acetate-2,2,4-trimethylpentane furnished needles, m.p. 72–73.5 °C; ^{1}H NMR ($CDCl_3$): δ 7.6–7.3 (m, 5 H), 5.58 (s, 1 H), 4.82 (s, 1 H), 4.5–3.8 (m, 4 H), 3.36 (s, 3 H), 2.66 (t, 1 H, *J* 7.8 Hz), 1.8–1.2 (m, 18 H), 0.88 (t, 3 H); ^{13}C NMR ($CDCl_3$): 201.0, 136.7, 129.2, 128.2, 126.5, 104.4, 102.3, 80.7, 69.6, 65.2, 57.2, 54.9, 31.9, 31.1, 29.5, 29.3, 27.3, 22.7, and 14.1 ppm; $[\alpha]_D^{20}$ +22.3° (*c* 1.0, CH_2Cl_2).

Synthesis of methyl 4,6-O-benzylidene-2-C-decyl-2-deoxy-3-C-[2-(1,3-dioxolan-2-yl)ethyl]-α-D-mannopyranoside (8) *by a Grignard reaction with* 6. A stock solution (1.38 M, THF) of the Grignard reagent derived from *10* was prepared according to Büchi and Wüest.[25] A solution of *6* (150 mg, 0.37 mmol) in THF (2 ml) was then added to 1.0 ml of this stock solution (22 °C). After 30 min TLC showed that practically all *6* had reacted and the mixture was then poured onto ice-aqueous ammonium chloride. Extraction with ether, drying ($MgSO_4$), and evaporation of the solvent gave a crude reaction product which according to HPLC analysis contained *7* (ret. time 6.7 min) and *8* (ret. time 3.5 min) in a ratio of about 1:6. The main product *8* was purified by flash chromatography using solvent system A–B (10:1). Part of *8* (80 mg) was obtained in chromatographically pure form as a colourless oil; combined yield of *7* and *8*: 120 mg (64 %). ^{1}H NMR ($CDCl_3$) of *8*: δ 7.6–7.3 (m, 5 H), 5.54 (s, 1 H), 4.92 (t, 1 H), 4.59 (s, 1 H), 4.2–3.6 (m, 8 H), 3.34 (s, 3 H) 2.64 (s, 1 H), 2.1–1.2 (m, 23 H), 0.89 (t, 3 H); ^{13}C NMR ($CDCl_3$): 137.8, 128.9, 128.1, 126.4, 104.9, 102.5, 102.3, 82.6, 71.5, 69.6, 64.8, 62.0, 55.1, 46.2, 31.9, 29.9, 29.7, 29.4, 28.0, 27.4, 26.3, 22.7, and 14.2 ppm.

Degradation of 8. Compound *8* (100 mg) was treated with acetic acid (1 ml), water (5 ml), and acetone (5 ml) at reflux (17 h). Solvents were evaporated, aqueous solutions of sodium hydroxide (0.5 M, 8 ml) and potassium permanganate (3 %, 20 ml) were added, and the mixture was left at 22 °C for 24 h. Acetone (1 ml) was added, and after 5 min the precipitate was filtered off and washed with water. Acidification with 2M hydrochloric acid to *ca.* pH 1, concentration to *ca.* 20 ml, extraction with ether (3×10 ml), drying the combined organic layers ($MgSO_4$), and evaporation of the solvent, gave a residue (52 mg) to which excess ethereal diazomethane was added. GLC analysis (OV-101, fused silica capillary column, 25 m) showed that the content of trimethyl decylhomocitrate was *ca.* 25 %; preparative HPLC afforded 8 mg. The HPLC retention time (2.9 min) differed clearly from that of the trimethyl ester prepared from authentic *1* (3.7 min), solvent system A–B (7:1), 3.5 ml/min. ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3 H), 3.67 (s, 6 H), 3.60 (d, 1 H, *J* 1.5 Hz), 2.77 (dd, 1 H, *J* 5.9 and 8.3 Hz), 2.6–1.1 (m, 22 H), 0.88 (t, 3 H).

Synthesis of methyl 4,6-O-benzylidene-2-C-decyl-2-deoxy-3-C-[2-(1,3-dioxolan-2-yl)ethyl]-α-D-altropyranoside (7) *by reaction of* 6 *with organolithium reagent* 11. A radical anion was first prepared at 0 °C in THF (100 ml) from lithium metal (30 mmol) and 4,4′-di-*tert*-butylbiphenyl (33 mmol) as described by Freeman and Hutchinson.[28] The solution was cooled to −60 to −65 °C and kept at that temperature during the addition (30 min) of a solution of bromide *10*[25] (1.8 g, 10 mmol) in THF (3 ml). When all *10* had been added, the mixture was stirred for an additional 5 min. A solution of *6* (1.6 g, 4.0 mmol) in THF (3 ml) was added in one portion and after 15 min (*ca.* −60 °C), the reaction mixture was poured onto saturated aqueous ammonium chloride (100 ml). Extraction with ether, drying ($MgSO_4$) and evaporation of the

solvent gave a crude mixture which according to HPLC analysis contained *7* and *8* in the approximate ratio 8:1. Flash chromatography using solvent system A–B (7:2) to which 0.1 % pyridine had been added, first gave a mixture (0.25 g) of *7* and *8* and then pure *7* (1.40 g) which crystallized; combined yield of *7* and *8*, 82 %. After recrystallisation from 2,2,4-trimethylpentane, *7* showed m.p. 79–80 °C; $[\alpha]_D^{20}$ −1.6° (*c* 1.0, CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 7.6–7.3 (m, 5 H), 5.53 (s, 1 H), 4.77 (dd, 1 H), 4.70 (s, 1 H), 4.4–3.5 (m, 9 H), 3.43 (s, 3 H), 2.0–1.2 (m, 23 H), 0.89 (t, 3 H); ^{13}C NMR ($CDCl_3$): 137.7, 128.8, 128.1, 126.3, 105.0, 102.2, 101.8, 81.0, 72.7, 69.3, 64.8, 60.1, 55.4, 46.8, 31.9, 30.1, 29.6, 29.4, 28.7, 28.5, 28.0, 27.5, 26.6, 26.3, 22.7, and 14.1 ppm.

Degradation of 7 into spiculisporic acid. A mixture of *7* (0.43 g), acetic acid (40 ml) and water (8 ml) was left at 22 °C (24 h). The mixture was concentrated at *ca.* 100 Pa, finally, by codistillation with toluene (3×30 ml, 30 °C). Treatment of the crude product with an excess of sodium borohydride in methanol (1.5 h, 22 °C), addition of acetic acid (1 ml), repeated codistillation with methanol, dissolution in chloroform, filtration, and evaporation of the solvent gave a product (0.34 g) which showed a single spot on TLC. Its ^{13}C NMR spectrum showed a glycosidic linkage but not the other two acetal functions. This material was further hydrolysed with acetic acid (20 ml) and water (4 ml) at reflux (30 min). Evaporation of the solvents, treatment with potassium permanganate (18 h) and work-up as described above for *8* yielded a crude trimethyl ester (183 mg), GLC purity *ca.* 20 %. Purification on silica gel, solvent system A–B (7:2), gave a trimethyl ester (47 mg) which was indistinguishable from the trimethyl ester prepared from *1* (HPLC retention time 3.7 min at conditions given for the isomer, ^{1}H NMR). ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 3 H), 3.73 (s, 3 H), 3.67 (s, 3 H), 3.63 (s, 1 H), 3.0–1.1 (m, 23 H), 0.88 (t, 3 H).

Hydrolysis (0.5 M NaOH, 22 °C, 40 h) of the trimethyl ester, acidification, extraction with ether, drying ($MgSO_4$), evaporation of the solvent, and drying (*ca.* 60 °C) of the residue over P_2O_5 at *ca.* 1 Pa (3 d) gave crystalline *1*, m.p. 143–6 °C, $[\alpha]_D^{20}$ −13.2° (*c* 0.6, ethanol); lit.[1] m.p. 145–6 °C, lit.[3] rotation $[\alpha]_D^{20}$ −13.4° (*c* 5.0, ethanol).

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