Personal Computer for data storage and manipulation. The quality of the data fit was judged by the χ^2 value.[28] The data are shown in Table II.

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Isolation of Acetylenic Sterols from a Higher Plant. Further Evidence That Marine Sterols Are Not Unique

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(24*R*)-5α-Stigmast-7-en-22-yn-3β-ol, 24,24-dimethyl-5α-cholest-7-en-22-yn-3β-ol, and 24,24-dimethyl-5α-cholesta-7,25-dien-22-yn-3β-ol were isolated from *Gynostemma pentaphyllum* (Cucurbitaceae). Their structures were determined by chemical and spectroscopic methods. They are the first acetylenic sterols isolated from a nonmarine organism. It is predicted that most of the sterols, which are now considered typical marine, will eventually also be found in freshwater organisms and terrestrial plants.

The isolation and structure of two sterols with a cyclopropyl group in the side chain, gorgosterol and 23-demethylgorgosterol, were reported in 1970.[4] Approximately 200 previously unknown sterols have since been isolated from marine organisms.[5] Sponges were the most interesting source of new compounds, which included classes of sterols that have not been found in marine organisms belonging to other phyla, viz. sterols with quaternary sp^3 carbons at C24, C25, or C26,[6] with methyl groups at C26 or C27 or both, and acetylenic sterols.[7]

It has been stated in several papers that marine sterols are unusual or unique.[5] However, this ignores the fact that until recently only sterol mixtures from marine sources have been carefully investigated by using modern separation techniques and analytical instruments.

Many phyla, such as Porifera[8] and Pyrrophyta, are not exclusively marine. The only known C_{28} sterol alkylated at C22 has actually been isolated from a freshwater or brackish water sponge (phylum Porifera).[9,10] Dinoflagellates (unicellular algae, phylum Pyrrophyta) have been shown to be primary producers of sterols with the gorgosterol side chain.[11,12] It will be only a matter of time before such cyclopropyl sterols are detected in freshwater algae belonging to the same phylum.

In the last 2 decades research on lipids from terrestrial plants has resulted in several papers on steroidal triterpenoids with a quaternary sp^3 carbon in the side chain[16] or a methyl group at C26 or both.[17] This strongly suggested that regular sterols with the same side chains would also be present in plants.[18] Such side chains were sup-

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Table I. ^{1}H NMR Spectral Data (400 MHz, $CDCl_3$) of the Acetates of the New Acetylenic Sterols (1, 5, 6), of Two Reduction Products (2, 7), and of Two Reference Compounds (3, 8)[a,b]

	1-acetate	2-acetate	3-acetate	5-acetate	6-acetate	7-acetate	8-acetate
C3-H	4.69 (m)	4.69 (m)	4.69 (m)	4.69 (m)	4.69 (m)	4.69 (m)	4.69 (m)
C6-H_2	1.79*			1.77*	1.78*		
C17-H	1.44*			1.43*	1.44*		
C18-H_3	0.564 (s)	0.563 (s)	0.548 (s)	0.559 (s)	0.558 (s)	0.562 (s)	0.544 (s)
C19-H_3	0.812 (s)	0.816 (s)	0.815 (s)	0.811 (s)	0.811 (d)	0.816 (s)	0.812 (s)
C20-H	2.472 (qdd) (7.2, 7.4, 2.1)	2.372 (m)		2.422 (qd) (6.6, 6.6)	2.468 (qd) (7.1, 7.1)	2.653 (qdd) (7.4, 6.6, 9.4)	
C21-H_3	1.189 (d, 6.8)	0.974 (d, 6.6)	1.031 (d, 6.6)	1.168 (d, 6.8)	1.170 (d, 6.8)	0.983 (d, 6.6)	1.009 (d, 7.4)
C22-H		5.228 (dd) (10.8, 10.4)	5.169 (dd) (14.8, 8.2)			4.987 (dd) (12.0, 9.9)	5.094 (dd) (15.4, 8.2)
C23-H		4.983 (dd) (11.2, 11.0)	5.032 (dd) (15.4, 8.8)			5.053 (d, 12.1)	5.273 (d, 15.4)
C24-H	2.07*						
C25-H	1.64*			1.49*			
C26-H_3 or -H_2	0.917 (d, 6.8)	0.853 (d, 7.2)	0.864 (d, 6.6)	0.933 (d, 6.6)	4.723 (1 H, s) 4.994 (1 H, s)	0.853 (d, 6.6)	0.804 (d, 6.6)
C27-H_3	0.944 (d, 6.8)	0.884 (d, 7.1)	0.794 (d, 6.6)	0.933 (d, 6.6)	1.834 (s)	0.853 (d, 6.6)	0.806 (d, 6.6)
C28-H_3	1.37*			1.100 (s)	1.298 (s)	1.036 (s)	0.891 (s)
C29-H_3	0.976 (t, 7.3)	0.829 (t, 7.2)	0.818 (t, 7.2)	1.100 (s)	1.298 (s)	1.049 (s)	0.893 (s)
acetate methyl	2.03 (s)	2.03 (s)	2.03 (s)	2.03 (s)	2.03 (s)	2.03 (s)	2.03 (s)

[a] Shifts are δ values. Internal standard TMS. The numbers in parentheses are splitting constants (Hz). Further signal at δ 5.15 (1 H, m, C7-H) was observed for all steryl acetates. [b] Asterisk denotes shifts determined by a COSY experiment.

posed to be typical of marine sponge sterols.

Recently, the occurrence of unprecedented (5β,19)-cyclo-14-methyl sterols in *Nervilia purpurea* (Orchidaceae) has been reported.[19] The same plant contains 24-isopropyl sterols,[20] which were first isolated from a sponge from the Great Barrier Reef.[21]

We already reported the isolation of five 24,24-dimethyl sterols[22] including **8**, **9**, and **10** from *Gynostemma pentaphyllum* (Cucurbitaceae) and of two 24-methylene-25-methyl sterols[23] from other plants of the same family. In this paper we describe the isolation and structure determination of several other new marine sponge type sterols that occur as minor sterols in *G. pentaphyllum*. They are the first examples of acetylenic sterols found in terrestrial plants.

Results and Discussion

Only 80% of the sterols of the aerial parts of *G. pentaphyllum* had been identified when we published an inventory of sterols of the Cucurbitaceae in 1986.[24] (22*E*,24*R*)-5α-Stigmasta-7,22-dien-3β-ol (**3**) and its 24*S* epimer are the predominant sterols (ratio >9:1, together 61.9%).[24,25] Our continuing work on the identification of its many minor and trace sterols[22,23,26] has led to the dis-

Chart I

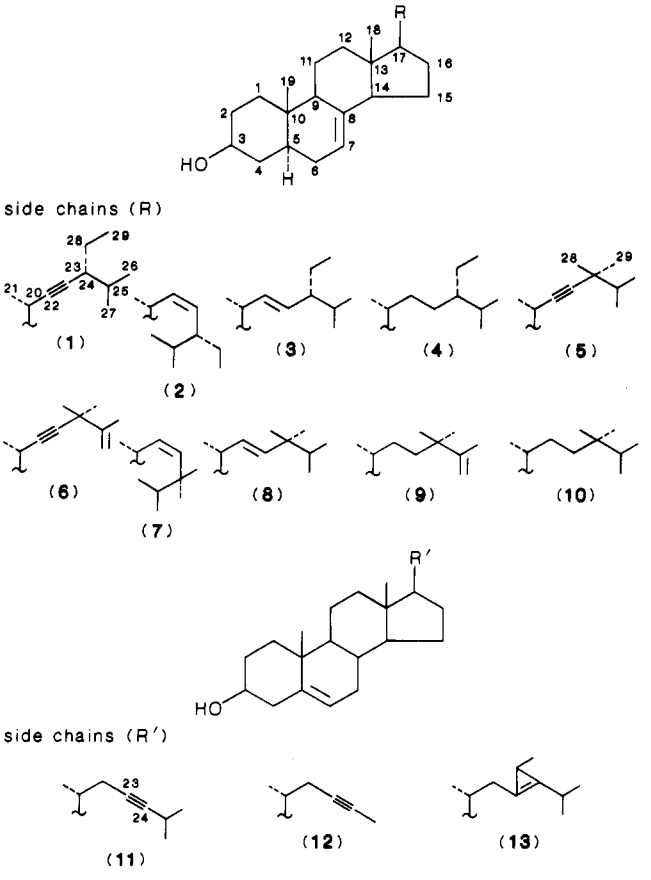

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Table II. ^{13}C NMR Spectral Data (100.62 MHz, $CDCl_3$) of the Acetates of the New Acetylenic Sterols (1, 5, 6) and of a Reduction Product (10)[a]

carbon	1-acetate	5-acetate	6-acetate	10-acetate
C1	26.85 (t)	36.89 (t)	36.81 (t)	36.82 (t)
C2	27.51 (t)	27.43 (t)	27.48 (t)	27.49 (t)
C3	73.39 (d)	73.44 (d)	73.44 (d)	73.48 (d)
C4	33.83 (t)	33.79 (t)	33.79 (t)	33.80 (t)
C5	40.12 (d)	40.02 (d)	40.07 (d)	40.04 (d)
C6	29.51 (t)	29.48 (t)	29.48 (t)	29.52 (t)
C7	117.35 (d)	117.41 (d)	117.47 (d)	117.27 (d)
C8	139.25 (s)	139.23 (s)	139.29 (s)	139.56 (s)
C9	49.30 (d)	29.25 (d)	49.24 (d)	49.25 (d)
C10	34.26 (s)	34.23 (s)	34.24 (s)	34.19 (s)
C11	21.32 (t)	21.27 (t)	21.27 (t)	21.47 (t)
C12	38.71 (t)	38.62 (t)	38.64 (t)	39.46 (t)
C13	43.28 (s)	43.28 (s)	43.29 (s)	43.34 (s)
C14	54.66 (d)	54.60 (d)	54.61 (d)	54.98 (d)
C15	22.94 (t)	22.90 (t)	22.89 (t)	22.95 (t)
C16	26.15† (t)	26.44 (t)	26.50 (t)	27.91 (t)
C17	56.01 (d)	55.97 (d)	55.89 (d)	55.91 (d)
C18	142.36* (q)	12.83 (q)	12.35 (q)	11.83 (q)
C19	12.94* (q)	12.92 (q)	12.93 (q)	12.93 (q)
C20	27.79 (d)	27.48 (d)	27.56 (d)	26.89 (d)
C21	21.26 (q)	22.22 (q)	21.93 (q)	19.00 (q)
C22	82.19# (s)	84.86* (s)	85.44* (s)	36.72† (t)
C23	86.89# (s)	87.15* (s)	86.95* (s)	29.35† (t)
C24	40.85 (d)	34.79 (s)	36.45 (s)	34.81 (s)
C25	31.51 (d)	37.76 (d)	150.31 (s)	34.96 (d)
C26	18.51@ (q)	19.39 (q)	19.42 (q)	17.40* (q)
C27	22.27@ (q)	18.39 (q)	108.92 (t)	17.42* (q)
C28	26.74† (t)	27.40 (q)	29.03 (q)	24.09# (q)
C29	12.42 (q)	27.40 (q)	29.03 (q)	24.18# (q)
CO*Me*	21.42 (q)	21.47 (q)	21.49 (q)	21.47 (q)
*C*OMe	170.63 (s)	170.70 (s)	170.72 (s)	170.70 (s)

[a] Internal standard TMS. Shifts are δ values. The symbols *, #, @, and † denote interchangeable assignments within a column. Multiplicities were determined by DEPT or INEPT experiments.

covery of new acetylenic sterols that were isolated by argentic TLC and reverse-phase HPLC. Their isolated yield is 0.01–0.03% of the sterols of *G. pentaphyllum*.

Structure Determination of the New Sterols

The acetates of **1** and **5** showed a molecular ion at m/z 452 ($C_{31}H_{48}O_2$) in their mass spectra, whereas the molecular ion of the acetate of **6** had m/z 450 ($C_{31}H_{46}O_2$). Thus all three compounds were C_{29} sterols, **1** and **5** having 3 degrees of unsaturation and **6** having 4 degrees of unsaturation. The mass spectra of all three sterols included prominent fragment ions at m/z 315 (loss of side chain) and 313 (loss of side chain +2H)[27] indicative of a regular monounsaturated sterol acetate skeleton. Comparison of the ^1H NMR data (Table I) with those of other sterols from the same source[22,24,26] left no doubt that the nuclear double bond was in the 7-position (Chart I).

This meant that **1** had 2 degrees of unsaturation in its side chain. The absence of olefinic side chain protons in the ^1H NMR spectrum and the presence of two quaternary side-chain carbons in the ^{13}C NMR spectrum at δ 82.19 and 86.19 (Table II) seemed to indicate the presence of a triple bond.[28] Quaternary carbons with similar shifts were also present in the ^{13}C NMR spectra of the acetates of **5** and **6** (Table II). The basic structure of the side chain of **1** was established by catalytic hydrogenation. The products were (24*S*)-5α-stigmast-7-en-3β-ol (**4**) acetate, which was readily identified on the basis of its ^1H NMR data,[24] and previously unreported (22*Z*,24*R*)-5α-stigmasta-7,22-dien-3β-ol (**2**) acetate (vide infra). Thus the structure of **1** is (24*R*)-5α-stigmast-7-en-22-yn-3β-ol. This assignment is further supported by the mass spectrum of its acetate, which includes strong peaks of fragments at m/z 409 ($M^+ - C_3H_7$ (C25–C27)), characteristic of Δ^{22}-unsaturated 24-alkyl sterols,[27,29] and at m/z 367 ($M^+ - C_6H_{13}$ (C24–C29)). All side-chain proton signals (Table I) were assigned by using the results of COSY and decoupling experiments (see Experimental Section for details). Interestingly, irradiation at δ 2.07 (C24-H) simplified the multiplet at δ 2.472 (qdd, C20-H) into a qd system (J = 7.1, 7.1 Hz) by eliminating a ^5J coupling ($J \sim 2$ Hz) across the triple bond (HCC≡CCH).[30]

The expected product of partial reduction of the triple bond[31] in **1**-acetate is (22*Z*,24*R*)-5α-stigmasta-7,22-dien-3β-ol (**2**) acetate. The compound we isolated showed the same fragmentation pattern in its mass spectrum as the acetate of (22*E*,24*R*)-5α-stigmasta-7,22-dien-3β-ol (**3**), which is the main sterol of *G. pentaphyllum*, but its ^1H NMR spectrum was different. C22-H and C23-H showed an ~11 Hz coupling (Table I), indicative of a cis configuration at the double bond. In contrast, the coupling between C22-H and C23-H in **3** is ~15 Hz (Table I). **2**-acetate could be converted into **4**-acetate by further reduction.

After the structure of **1** had been determined we were sure that the other two new sterols (**5**, **6**) were also Δ^7 sterols with a triple bond in the side chain because of spectral similarities (notably the presence of two quaternary carbons having δ 82–87 in the ^{13}C NMR spectrum (Table II)) and because the corresponding acetylated sterols had been isolated from the same fraction of argentic TLC (their very low R_f value, apparently, being indicative of the presence of a triple bond). Thus the remaining problem was the determination of the substitution pattern of the side chains of **5** and **6**, which have two more carbon atoms than the cholesterol side chain.

The acetate of **5** gave two products (**7**, **10**) on catalytic reduction. **10**-acetate was identified by NMR comparison[22a] as 24,24-dimethyl-5α-cholest-7-en-3β-ol acetate (**10** occurs as a minor sterol in *G. pentaphyllum*[22a]). Thus the structure of **5** is 24,24-dimethyl-5α-cholest-7-en-22-yn-3β-ol. The mass spectrum of the other hydrogenation product (**7**-acetate, MW 454, $C_{31}H_{50}O_2$), which has 2 degrees of unsaturation, showed the same fragmentation pattern as (22*E*)-24,24-dimethyl-5α-cholesta-7,22-dien-3β-ol (**8**) acetate (**8** is one on the minor sterols of *G. pentaphyllum*[22a]). Further hydrogenation of **7**-acetate afforded **10**-acetate. A comparison of the ^1H NMR spectra of **7**- and **8**-acetate (Table I) confirmed that these compounds are double-bond isomers, thus **7** is (22*Z*)-24,24-dimethyl-5α-cholesta-7,22-dien-3β-ol.

The third new sterol (**6**) had 3 degrees of unsaturation in the side chain. Because of the presence in the ^1H NMR spectrum of its acetate (Table I) of an olefinic methyl (δ 1.834), two methylene protons (δ 4.723, 4.994) and two magnetically equivalent methyl groups (singlets, δ 1.298) in addition to a methyl doublet (δ 1.170), the only possible structure for **6** was 24,24-dimethyl-5α-cholesta-7,25-dien-22-yn-3β-ol. As expected, partial hydrogenation of **6**-acetate afforded a mixture of the acetates of **7** and **10**.

The only other known acetylenic sterols (**11**, **12**) were isolated from the Mediterranean sponge *Calyx niceaensis*.[7]

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They are anomalous side-chain sterols (i.e. sterols with less carbon atoms in the side chain than cholesterol), which are biosynthetically unrelated to the new *G. pentaphyllum* sterols (**1**, **5**, **6**). The results of incorporation experiments[32] with 24-methylenecholesterol-*28*-^{14}C suggest that **11** and **12** are catabolites (fragmentation products?) of calysterol[33] (**13**), the major sterol of *C. niceaensis*, or of one of its double-bond isomers.[34] It is known, mainly through the efforts of Goad et al. in Goodwin's group,[35] that Δ^{22}-unsaturated side chains are formed by dehydrogenation of their saturated analogues. It seems likely that the acetylenic sterols of *G. pentaphyllum* arose by further dehydrogenation of Δ^{22} precursors which occur in the same plant.[22a]

The only reason why so many marine sterols were found is that a major effort was made to find them[36] and that the separation techniques and instrumentation have greatly improved since the 1950's when much work on plant sterols was done.[37] The same techniques have been applied to *Nervilia purpurea* by Kikuchi et al.[19,20] and by us to the Cucurbitaceae and other higher plants. The results have been rewarding. Indications are that terrestrial plants will prove to be treasure houses of sterols and that the distinction between marine sterols and freshwater and terrestrial sterols will gradually fade and eventually disappear.

Experimental Section

General Methods. Melting points are uncorrected. Argentation TLC plates (silica gel–$AgNO_3$, 4:1) were developed three times with 5:1 CCl_4–CH_2Cl_2. HPLC separations were performed on a Whatman Partisil 5 ODS-2 column (25 cm × 10 mm i.d.) with methanol as the mobile phase (flow 4 mL/min) and a refractive index detector. A SCOT OV-17 glass capillary column was used for GC (30 m × 0.3 mm i.d., 260 °C). Cholesterol acetate was the standard for the determination of relative retention times in both GC and HPLC (RRT = 1.00). The EI/MS spectra were taken on a Hitachi M-80B double focussing GC/MS (70 eV, direct probe) instrument. All ^1H NMR spectra (400 MHz) were recorded on JEOL spectrometers. The ^{13}C NMR spectra (100.62 MHz) were run on a JEOL instrument and a Bruker AM400 instrument. Acetylation was performed in Ac_2O–pyridine at room temperature overnight. Hydrogenation was carried out in EtOH by using prereduced PtO_2 at atmospheric pressure and at room temperature. The dried aerial parts of *G. pentaphyllum* were purchased from Kinokuniya Kan-Yaku Kyoku Co. (Tokyo).

Extraction and Isolation. Air-dried aerial parts of *G. pentaphyllum* (20 kg) were extracted with CH_2Cl_2 (reflux, 7 h). The extract (580 g) was saponified (5% KOH in MeOH, reflux, 3 h). The residue was partitioned between water and isopropyl ether. Yield of neutral unsaponifiables: 107 g. They were fractionated over silica gel (700 g) using (1) hexane (2.5l), (2) hexane–ether, 9:1 (3.0l), (3) hexane–ether, 4:1 (2.5l), (4) hexane–EtOAc, 6:1 (9.0l), (5) hexane–EtOAc, 3:1 (2.5l), and (6) MeOH (2.0l). Free sterols (31 g) were obtained from the 6:1 hexane–EtOAc eluate. They were acetylated. The crude acetates (30 g) were recrystallized from acetone–MeOH. Yield: 18.3 g of crystals and 8.9 g of residue of the mother liquor. This residue was purified over silica gel (200 g; eluent hexane–ether, 4:1) to give 2.0 g of acetates, which were fractionated by argentic TLC. Seven bands were scraped and extracted. The fraction obtained from the most polar band (186 mg, R_f 0.05–0.11) was worked up by reverse-phase HPLC and afforded the acetates of **1** (6.5 mg), **5** (2.7 mg), and **6** (2.7 mg).

(24*R*)-5α-Stigmast-7-en-22-yn-3β-ol (1) acetate: mp 128–130 °C; RRT(GC) 1.60, RRT(HPLC) 0.49; MS, *m/z* (assignment, relative intensity) 452.3669 ($C_{31}H_{48}O_2$, M^+, 41, calcd 452.3652), 437.3422 ($C_{30}H_{45}O_2$, 18), 409.3083 ($C_{28}H_{41}O_2$, 26), 377.3151 ($C_{28}H_{41}$, 4), 367.2634 ($C_{25}H_{35}O_2$, 9), 349.2907 ($C_{26}H_{37}$, 10), 315.2268 ($C_{21}H_{31}O_2$, 3), 299.2059 ($C_{20}H_{27}O_2$, 5), 255.2102 ($C_{19}H_{27}$, 59), 241.1995 ($C_{18}H_{25}$, 7), 239.1812 ($C_{18}H_{23}$, 7), 229.1980 ($C_{17}H_{25}$, 23), 213.1667 ($C_{16}H_{21}$, 13), 43.0182 ($C_2H_3O_1$, 100). Hydrogenation of this acetate (4 h) afforded a mixture of the acetates of **2** (80%) and **4** (20%), which was separated by HPLC.

Decoupling experiments: Irradiation at δ 0.917 (3 H, d) simplified the multiplet at δ 1.63 (1 H) and irradiation at δ 1.63 collapsed the doublets at δ 0.917 and 0.944 (3 H) and simplified the multiplet at δ 2.07 (1 H). This allowed the assignment of the methyl doublets to C26-H and C27-H and of the two methine multiplets to C25-H and C24-H, respectively. Further irradiation at δ 1.189 (3 H, d) collapsed the multiplet at δ 2.472 (1 H, qdd) into a double doublet (J = 2.0, 7.5), whereas irradiation at δ 2.07 collapsed the signal at δ 2.472 (1 H, qdd) into a quadruple doublet (J = 7.1, 7.1) and simplified the multiplet at δ 1.63 (C25-H). This allowed the assignment of the methine multiplet at δ 2.472 to C20-H and of the methyl doublet at δ 1.189 to C21-H.

(22*Z*,24*R*)-5α-Stigmasta-7,22-dien-3β-ol (2) acetate: mp 138–139 °C; RRT(GC) 1.68, RRT(HPLC) 0.88.

Decoupling experiments: Irradiation at δ 0.974 (3 H, d) simplified the multplet at δ 2.372 (1 H), whereas irradiation at δ 2.372 collapsed the doublet at δ 0.974 and the signal at δ 5.228 (1 H, dd) into a doublet (J = 11.0). Further irradiation at δ 5.228 collapsed the double doublet at δ 4.983 (1 H) into a doublet (J = 10.0) and simplified the methine multiplet at δ 2.372. This allowed assignment of the signals at δ 0.974, 2.372, 4.983, and 5.228 to C21-H, C20-H, C23-H, and C22-H, respectively. The remaining two methyl doublets and the methyl triplet were assigned to C26-H, C27-H, and C29-H, respectively.

(24*S*)-5α-Stigmast-7-en-3β-ol (4) acetate: mp 157–160 °C; RRT(GC) 1.94, RRT(HPLC) 1.18. The configuration at C24 of this reduction product of **1** was determined by ^{13}C NMR in $CDCl_3$. Comparison of the following data with literature data for an authentic compound[24] and another sterol[38] with the same side chain proves that **1** and **4** have the 24*S*/β-configuration. δ (assignment): 36.82 (C1), 27.48 (C2), 73.47 (C3), 33.79* (C4), 40.04 (C5), 29.52 (C6), 117.26 (C7), 139.55 (C8), 49.25 (C9), 34.18 (C10), 21.46 (C11), 39.47 (C12), 43.34 (C13), 54.97 (C14), 22.94# (C15), 27.94 (C16), 56.02 (C17), 11.84 (C18), 12.93 (C19), 36.71 (C20), 18.94 (C21), 33.82* (C22), 26.47 (C23), 46.03 (C24), 28.92 (C25), 19.56 (C26), 18.94 (C27), 22.97# (C28), 12.31 (C29), 21.46 (acetate methyl), 170.63 (acetate carbonyl) [*, # denote interchangeable assignments]. ^1H NMR data ($CDCl_3$). Assignment, δ (multiplicity, J in Hz): C3-H, 4.69 (m); C18-H_3, 0.533 (s); C19-H_3, 0.810 (s); C21-H_3, 0.930 (d, 6.1); C26-H_3, 0.812 (d, 6.7); C27-H_3, 0.831 (d, 7.0); C29-H_3 (t, 7.3); acetate CH_3, 2.03 (s).

24,24-Dimethyl-5α-cholest-7-en-22-yn-3β-ol (5) acetate: mp 149–151 °C; RRT(GC) 1.41, RRT(HPLC) 0.45; MS, *m/z* (assignment, relative intensity) 452.3669 ($C_{31}H_{48}O_2$, M^+, 41, calcd 452.3652), 437.3422 ($C_{30}H_{45}O_2$, 18), 409.3083 ($C_{28}H_{41}O_2$, 26), 377.3151 ($C_{28}H_{41}$, 4) 367.2634 ($C_{25}H_{34}O_2$, 9), 353.2482 ($C_{24}H_{33}O_2$, 5), 349.2907 ($C_{26}H_{37}$, 10), 315.2268 ($C_{21}H_{31}O_2$, 30), 313.2146 ($C_{21}H_{29}O_2$, 42), 300.2097 ($C_{20}H_{28}O_2$, 5), 299.2059 ($C_{20}H_{27}O_2$, 5), 255.2102 ($C_{19}H_{27}$, 27), 253.1948 ($C_{19}H_{25}$, 11), 241.1995 ($C_{18}H_{25}$, 7), 239.1812 ($C_{18}H_{23}$, 7), 229.1980 ($C_{17}H_{25}$, 23), 213.1667 ($C_{16}H_{21}$, 13), 43.0182 ($C_2H_3O_1$, 100). Hydrogenation of **5**-acetate (4 h) afforded a mixture of the acetates of **7** (70%) and **10** (30%), which was separated by HPLC.

Decoupling experiments: Irradiation at δ 1.161 (3 H, d) collapsed the signal at δ 2.422 (1 H, qd) into a doublet (J = 6.6), whereas irradiation at δ 2.422 collapsed the methyl doublet at δ

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1.161. This allowed assignment of the signals at δ 1.161 and 2.422 to C21-H and C20-H, respectively. This means that the remaining highly deshielded dimethyl singlet (δ 1.100) must be caused by C28-H and C29-H and the dimethyl doublet (δ 0.933) by C26-H and C27-H.

24,24-Dimethyl-5α-cholesta-7,25-dien-22-yn-3β-ol (6) acetate: mp 138–140 °C; RRT(GC) 1.50, RRT(HPLC) 0.37; MS, *m/z* (assignment, relative intensity) 450.3461 ($C_{31}H_{46}O_2$, M^+, 34, calcd 450.3494), 435.3247 ($C_{30}H_{43}O_2$, 12), 407.2922 ($C_{28}H_{39}O_2$, 4), 475.3064 ($C_{28}H_{39}$, 4), 367.2634 ($C_{25}H_{35}O_2$, 4), 315.2286 ($C_{21}H_{31}O_2$, 18), 313,2114 ($C_{21}H_{29}O_2$, 10), 299.2046 ($C_{20}H_{27}O_2$, 6), 267.2071 ($C_{20}H_{27}$, 2), 255.2102 ($C_{19}H_{27}$, 38), 253.1945 ($C_{19}H_{25}$, 6), 239.1843 ($C_{18}H_{23}$, 6), 229.0000 ($C_{17}H_{25}$, 11), 213.1657 ($C_{16}H_{21}$, 9), 43.0193 ($C_2H_3O_1$, 100). Hydrogenation of **6**-acetate (4 h) afforded a mixture of the acetates of **7** (70%) and **10** (30%), which was separated by HPLC. The MS and ^1H NMR data of these acetates were indistinguishable from those of the hydrogenation products of **5**-acetate.

Decoupling experiments: Irradiation of the methyl doublet at δ 1.170 collapsed the signal at δ 2.468 (1 H, qd) into a doublet (J = 6.8) and irradiation at δ 2.468 collapsed the methyl doublet at δ 1.170. Thus we found C20-H and C22-H.

(22*Z*)-24,24-Dimethyl-5α-cholesta-7,22-dien-3β-ol (7) acetate: mp 195–197 °C; RRT(GC) 1.88, RRT(HPLC) 0.90; MS, *m/z* (assignment, relative intensity) 454.3804 ($C_{31}H_{50}O_2$, M^+, 54), 439.3545 ($C_{30}H_{47}O_2$, 18), 411.3237 ($C_{28}H_{43}O_2$, 18), 394.3556 ($C_{29}H_{46}$, 8), 351.3080 ($C_{26}H_{39}$, 9), 342.2587 ($C_{23}H_{34}O_2$, 14), 315.2308 ($C_{21}H_{31}O_2$, 30), 313.2131 ($C_{21}H_{29}O_2$, 75), 299.2018 ($C_{20}H_{27}O_2$, 7), 288.2065 ($C_{19}H_{28}O_2$, 20), 273.1856 ($C_{18}H_{25}O_2$, 8), 255.2081 ($C_{19}H_{27}$, 67), 253.1962 ($C_{19}H_{25}$, 7), 241.1967 ($C_{18}H_{25}$, 9), 229.1937 ($C_{17}H_{25}$, 37), 213.1684 ($C_{16}H_{21}$, 20), 81.0717 (C_6H_9, 100).

Decoupling experiments: Irradiation at δ 0.983 collapsed the methine signal at δ 2.653 (qdd) into a double doublet (J = 9.6, 7.4), whereas irradiation at δ 2.653 collapsed the methyl doublet at δ 0.983 into a singlet and the methine signal at δ 4.987 (dd) into a doublet (J = 12.5). Further irradiation at δ 4.987 (1 H, dd) collapsed the methine signal at δ 2.653 (qdd) into a quadruple doublet (J = 6.6, 9.4) and the methine doublet at δ 5.053 into a singlet. On the basis of these results the signals at δ 0.983, 2.653, 4.987, and 5.053 were assigned to C21-H, C20-H, C22-H, and C23-H, respectively.

24,24-Dimethyl-5α-cholest-7-en-3β-ol (10) acetate: mp 180–183 °C; RRT(GC) 2.02, RRT(HPLC) 1.22; MS, *m/z* (relative intensity) 456 (M^+, 100), 441 (18), 413 (3), 396 (59), 381 (12), 315 (11), 288 (8), 273 (9), 255 (67), 229 (20), 213 (29). ^1H NMR data were reported earlier.[22a] The previously unreported ^{13}C NMR data are included in Table I. Assignment of the side-chain ^{13}C signals was made with the aid of ^{13}C NMR data for the relevant model paraffins.[39]

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Registry No. **1**, 118112-67-7; **1** acetate, 118112-68-8; **2**, 118203-79-5; **4**, 82467-98-9; **5**, 118112-69-9; **5** acetate, 118112-73-5; **6**, 118142-17-9; **6** acetate, 118112-71-3; **7**, 118112-70-2; **7** acetate, 118112-72-4; **10**, 105097-81-2; **10** acetate, 105097-84-5.

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C-Glycosides. 7.† Stereospecific C-Glycosylation of Aromatic and Heterocyclic Rings

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Stereospecific C-glycosylation of aromatic and heterocyclic rings can be realized by reacting the corresponding organolithium derivatives with benzylated lactones and reducing the so-obtained lactols with triethylsilane in the presence of boron trifluoride etherate at low temperature. Debenzylation proceeds without opening of the ring in pyrano series, but with opening in furano series.

Because of their biological importance, considerable effort has been devoted toward the synthesis of C-glycosides during recent years.[1]

With the goal of devising new and efficient methodologies for the stereocontrolled synthesis of this class of compounds, we are studying the reactivity of several sugar derivatives, diversely activated at the anomeric carbon atom, with a variety of organometallic reagents. Several combinations have already been studied and the results published: glycals with arylpalladium species,[2] peracylated enones[3] and protected 1,2-anhydro sugars[4a] with organocuprates.

We report herein our results concerning the reaction of organolithium derivatives with protected lactones and the reduction of the products so obtained into C-glycosides (Scheme I).

Sugar lactones have previously been employed for that purpose by Kishi et al.[5] to prepare allyl-C-glycosides. In their approach to C-nucleosides, Asbun and Binkley[6] and Ogura et al.[7] effected C–C bond formation in this fashion but were not successful in reduction of the lactol product.

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