Synthesis of a Triaromatic Steroid Biomarker, (20*R*,24*R*)-4,17β-Dimethyl-18,19-dinorstigmasta-1,3,5,7,9,11,13-heptaene, from Stigmasterol[1]

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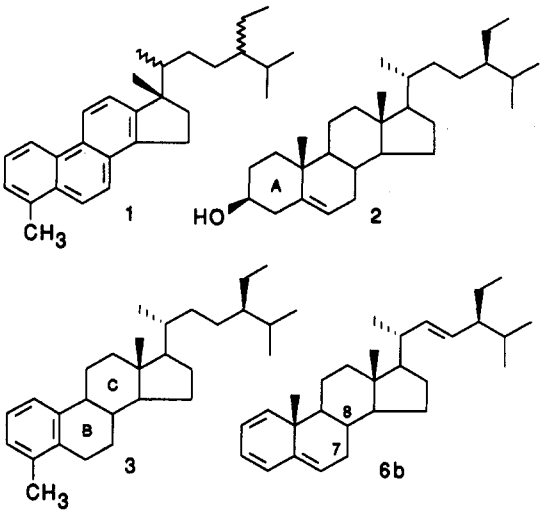
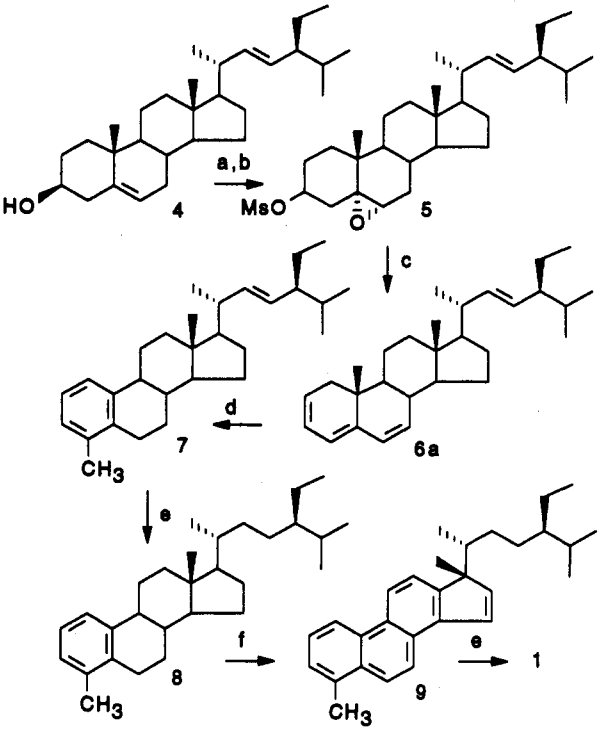
Triaromatic steroids serve as useful geochemical "biomarkers"[2] for assessing the maturation of petroleum and prompted our interest in a synthesis of selected diastereomers of the triaromatic stigmastane,[3] (20ζ,24ζ)-4,17β-dimethyl-18,19-dinorstigmasta-1,3,5,7,9,11,13-heptaene (**1**). Published routes[4] for synthesizing triaromatic steroids involve an initial aromatization of ring A as, for example, in the conversion of β-sitosterol (**2**) to the monoaromatic steroid **3** in Scheme 1. A subsequent aromatization of rings B and C in **3** provide the triaromatic steroid **1** in which the C-18 and C-19 angular methyl groups have migrated to the C-4 and C-17β positions, respectively. The presence of campesterol in many commercial samples of β-sitosterol (**2**) complicated, however, the preparation of a pure sample of the biomarker **1** using the literature procedure. In addition, in the case of (20*R*,22*E*,24*S*)-stigmasterol (**4**), this literature procedure suffered from particularly poor yields in the aromatization of the A ring. We developed a synthesis of (20*R*,24*R*)-4,17β-dimethyl-18,19-dinorstigmasta-1,3,5,7,9,11,13-heptaene (**1**) from (20*R*,22*E*,24*S*)-stigmasterol (**4**) that employed an improved, two-step aromatization of the A ring.

As shown in Scheme 2, the conversion of (22*E*)-stigmasterol (**4**) to 5α,6α-epoxy-3β-mesylate[5] **5** and heating **5** in hexamethylphosphoramide (HMPA) at 230 °C for 5 minutes led to the $\Delta^{2,4,6,22}$-tetraene **6a** in high yield. The assigment of the $\Delta^{2,4,6,22}$-tetraene structure **6a** rather than the isomeric $\Delta^{1,3,5,22}$-tetraene **6b** (Scheme 1) rested on a detailed analysis of the ^{1}H NMR spectrum (supplementary material). The ^{1}H NMR spectrum displayed the expected two-spin vinylic system for H-22 and H-23, a second two-spin vinylic system for H-6 and H-7, and finally a three-spin vinylic system for H-2, H-3, and H-4 consistent only with $\Delta^{2,4,6,22}$-tetraene **6a**. In addition, the allylic H-1 ethylene signals in ^{1}H COSY NMR of $\Delta^{2,4,6,22}$-tetraene **6a** showed geminal and allylic coupling to H-2 but no other coupling. If the corresponding isomeric $\Delta^{1,3,5,22}$-tetraene **6b** were the correct structure, the allylic H-7 methylene group would exhibit a similar geminal and allylic coupling but would also display an additional coupling to H-8. Finally, the chemical shift for the C-19 angular methyl signal in **6a** was consistent with values reported in the literature for a related system.[6]

Scheme 1



Scheme 2



[a] Key: (a) *m*-CPBA, CH_2Cl_2, 0 °C; (b) MsCl, Et_3N; (c) HMPA, 230 °C; (d) HBr, aqueous HOAc; (e) H_2, PtO_2; (f) chloranil.

(1) This is paper 8 in a series on the Synthesis of Biomarkers in Fossil Fuels. For paper 7 in this series, see: Stoilov, I.; Kolaczkowska, E.; St. Pyrek, J.; Watt, D. S.; Carlson, R. M. K.; Fago, F. J.; Moldowan, J. M. *J. Org. Chem.* **1993**, *58*, 3444.

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(5) Ogilvie, A. G.; Hanson, J. R. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1981. (b) Hanson, J. R.; Reese, P. B. *Tetrahedron Lett.* **1983**, *24*, 3405. (c) Hanson, J. R.; Reese, P. B.; Sadler, I. H. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2937. (d) Hanson, J. R.; Organ, T. D. *J. Chem. Soc. C* **1970**, 2473.

(6) (a) δ 0.71 (C-18 CH_3), 0.95 (C-19 CH_3) in $CDCl_3$ for (20*R*)-26-norcholestesta-2,4,6-trien-26-one: Moreau, J. P.; Aberhart, D. J.; Caspi, E. *J. Org. Chem.* **1974**, *39*, 2018. (b) No data given for (20*R*)-cholesta-2,4,6-triene: Kurasawa, Y.; Takeda, A.; Ueda, T. *Chem. Pharm. Bull. (Tokyo)* **1976**, *24*, 375.

0022-3263/94/1959-0926$04.50/0

Although the HMPA-mediated dehydration of alcohols[7] to olefins is well known, the conversion of an epoxide to a diene under similar conditions represented an interesting extension of this reaction. The further acid-catalyzed rearrangement of the $\Delta^{2,4,6,22}$-tetraene **6a** using hydrobromic acid in acetic acid provided the desired monoaromatic steroid **7** in 72% yield. The appearance of the C-4 methyl group at δ 2.21 in the ^{1}H NMR spectrum of **7** was consistent with literature reports[8] for related 4-methylsteroids but did not, at this stage, rigorously exclude the isomeric C-1 methyl analogs that also display the C-1 methyl at δ 2.2–2.3.[9] Ogilvie[5] has speculated that the direct acid-catalyzed rearrangement of the 5α,6α-epoxy-3β-mesylate of β-sitosterol (**2**) proceeded *via* a related $\Delta^{2,4,6}$-triene. The route in Scheme 2 lent support to this suggestion and offered an improvement in the overall yield for such A ring monoaromatization reactions.

The reduction of the Δ^{22}-olefin in the monoaromatic steroid **7** and a chloranil oxidation[4] of **8** furnished the (20*R*,24*R*)-4,17β-dimethyl-18,19-dinorstigmasta-1,3,5,7,9,-11,13,15-octaene (**9**). The C-4 methyl signal in **9** appeared at δ 2.77, and the C-17β angular methyl shifted from δ 0.70 in **8** to δ 1.41 in **9** consistent with literature reports.[3a,10] The chemical shift data also excluded the isomeric C-1 methyl analogs that might have arisen during the acid-catalyzed rearrangement of **6a** and that typically display the C-1 methyl at δ 3.1–3.2.[11] The hydrogenation of **9** using palladium on carbon in a related system led, contrary to a literature report,[4] to reduction of both the Δ^{22}-olefin and the B ring; however, a catalytic hydrogenation using platinum oxide led selectively to the desired biomarker **1**. This route for the synthesis of **1** from (22*E*)-stigmasterol (**4**) was preferable to the route from β-sitosterol (**2**) not only from the vantage point of overall yield but also from the perspective of the interesting conversion of the 5α,6α-epoxy-3β-mesylate **5** to the $\Delta^{2,4,6,22}$-tetraene **6a**. The scope and mechanism of this process are under investigation.

Experimental Section

(20*R*,22*E*,24*S*)-5α,6α-Epoxystigmast-22-en-3β-ol Methanesulfonate (5). To 10 g of (20*R*,22*E*,24*S*)-stigmasterol (**4**) in 350 mL of CH_2Cl_2 at 0 °C was added 6.7 g (38.3 mmol, 1.3 equiv) of *m*-chloroperoxybenzoic acid in several portions. The mixture was stirred at 0 °C for 1 h and filtered. The filtrate was washed successively with saturated $NaHCO_3$, H_2O, and brine. The solution was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford 8.5 g (82%) of crude (20*R*,22*E*,24*S*)-5α,6α-epoxystigmast-22-en-3β-ol[12] that was sufficiently pure to be used in the next reaction. A small sample of the crude product was purified by crystallization and had the following physical and spectral data: mp 149–51 °C; ^{13}C NMR ($CDCl_3$) δ 12.0 (C-18), 12.2 (C-14), 15.8, 20.5, 21.0, 21.1, 24.0, 25.3, 28.7, 29.7, 30.9, 31.8, 32.3, 34.8, 39.2, 40.4, 42.1, 51.1, 55.7, 59.2, 65.8, 68.5, 76.5, (CHOH), 128.7, 130.0.

To a solution of 8.5 g (19.8 mmol) of crude (20*R*,22*E*,24*S*)-5α,6α-epoxystigmast-22-en-3β-ol in 60 mL of CH_2Cl_2 at 0 °C under a N_2 atmosphere was added 8.4 mL (57.8 mmol, 3 equiv) of Et_3N followed by the slow addition of 2 mL (25.8 mmol, 1.3 equiv) of methanesulfonyl chloride. The mixture was stirred for 1 h at 0 °C and was diluted with 250 mL of cold water. The solution was extracted with CH_2Cl_2. The organic layer was washed successively with H_2O to neutral pH and with 50 mL of brine. The solution was dried over anhydrous $MgSO_4$, filtered, and concentrated to afford 9.6 g of crude **5**. A portion (409 mg) was chromatographed on silica gel using 1:3:1 EtOAc–hexane to give 289 mg of **5**: mp 162–164 °C; ^{13}C NMR ($CDCl_3$) δ 12.0 (C-18), 12.2 (C-19), 15.7, 18.9, 20.5, 21.0, 21.1, 24.0, 25.3, 28.5, 28.6, 29.7, 31.8, 32.1, 34.7, 37.2, 38.3, 39.1, 40.4, 42.1, 42.3, 51.1, 55.6, 59.2, 62.1, 63.4, 64.9, 79.6, 129.3, 138.1. Anal. Calcd for $C_{30}H_{50}SO_4H$: C, 71.09; H, 9.95. Found: C, 71.18; H, 9.93.

(20*R*,22*E*,24*S*)-Stigmasta-2,4,6,22-tetraene (6a). A solution of 67.1 g (132 mmol) of **5** in 350 mL of anhydrous HMPA was heated at 230 °C for 5 min under a N_2 atmosphere. The solution was poured into water and extracted with two 500-mL portions of hexane. The organic layer was washed successively with H_2O and brine and dried over anhydrous $MgSO_4$. The solution was filtered, concentrated, and crystallized from 1:1.2 EtOH–EtOAc to give 47.8 g (92%) of **6a** as white crystals: mp 99–102 °C (from EtOH–EtOAc); ^{13}C NMR ($CDCl_3$) δ 12.0 (C-18 CH_3), 12.2 (C-19 CH_3) 15.3, 19.0, 20.9, 21.1, 21.2, 23.8, 25.4, 28.9, 31.9, 35.6, 36.4, 37.0, 39.8, 40.5, 43.0, 51.2, 51.6, 54.7, 55.8, 119.2, 124.2, 125.2, 127.9, 129.4, 129.9, 131.6, 142.8. Anal. Calcd for $C_{29}H_{44}\cdot 1/2C_2H_5OH$: C, 86.67; H, 11.39. Found: C, 86.39; H, 11.23.

(20*R*,22*E*,24*S*)-4-Methylstigmasta-1,3,5(10),22-tetraene (7). A solution of 40.7 g (104 mmol) of **6a** in 170 mL of acetic acid and 76.2 mL (1.4 mol, 10 equiv) of 48% aqueous HBr was heated to reflux for 1 h. The solution was cooled, and the acid was neutralized with saturated $NaHCO_3$ solution. The solution was extracted with EtOAc. The organic solutions were washed successively with water and brine and dried over anhydrous $MgSO_4$. The solution was filtered and concentrated to yield 41 g of crude **7** that was chromatographed on alumina with hexane to yield 29.3 g (72%) of **7**: mp 116–118 °C; ^{13}C NMR ($CDCl_3$) δ 12.1, 12.2, 19.0, 19.8, 21.2, 24.0, 25.4, 26.8, 27.2, 27.8, 29.1, 32.0, 37.9, 39.9, 40.6, 42.5, 44.5, 51.3, 55.7, 56.2, 123.1 (C-1), 125.2 (C-2), 127.2 (C-3), 129.3, 135.2 (C-4), 136.3 (C-5), 138.3, 140.6 (C-10). Anal. Calcd for $C_{29}H_{44}$: C, 88.70; H, 11.29. Found: C, 88.77; H, 11.28.

(20*R*,24*R*)-4-Methylstigmasta-1,3,5(10)-triene (8).[3] A solution of 1.5 g (3.8 mmol) of **7** in 20 mL of 1:2 benzene–hexane and 863 mg (3.8 mmol, 1 equiv) of PtO_2 was hydrogenated at 60 psi for 4 h at 25 °C. The solution was filtered, concentrated, and chromatographed on silica gel using hexane to afford 1.34 g (89%) of **8**: mp 54–55 °C (from hexane); ^{13}C NMR ($CDCl_3$) δ 11.9, 12.0, 18.7, 19.0, 19.8, 23.0, 23.9, 26.0, 26.8, 27.2, 27.8, 28.3, 29.1, 33.9, 36.1, 37.9, 40.0, 42.6, 44.5, 45.8, 55.6, 56.3, 123.1 (C-1), 125.2 (C-2), 127.2 (C-3), 135.2 (C-4), 136.3 (C-5), 140.6 (C-10). Anal. Calcd for $C_{29}H_{46}$: C, 88.25; H, 11.75. Found: C, 88.03; H, 11.75.

(20*R*,24*R*)-4,17β-Dimethyl-18,19-dinorstigmast-1,3,5,7,9,-11,13,15-octaene (9).[3] To a solution of 1.34 g (3.39 mmol) of **8** dissolved in 25 mL of anisole was added 5.39 g (21.9 mmol, 6.65 equiv) of crystallized chloranil in three portions over a period of 30 h. The solution was refluxed for 30 h. The solution was filtered through alumina with hexane. The filtrate was concentrated and chromatographed on a silica gel column using hexane to give 210 mg (16%) of **9**. However, a trace impurity, detected by GC–MS, was present in this sample, and a portion of this product was subjected to a second chromatography on an analytical silica gel plate using hexane (two developments) to afford a pure sample of **9**: ^{13}C NMR ($CDCl_3$) δ 12.5, 12.8, 15.2, 19.6, 20.5, 22.2, 23.7, 29.1, 29.7, 31.7, 40.7, 46.2, 58.8, 120.6, 121.1, 121.6, 123.1, 123.4, 127.3, 128.8, 144.9, 151.6; exact mass spectrum calcd for $C_{29}H_{36}$ 384.2817, found 384.2824.

(20*R*,24*R*)-4,17β-Dimethyl-18,19-dinorstigmasta-1,3,5,7,9,-

(7) (a) Monson, R. S. *Tetrahedron Lett.* **1971**, *12*, 567. (b) Monson, R. S.; Preist, D. N. *J. Org. Chem.* **1971**, *36*, 3826. (c) Lomas, J. S.; Sagatys, D. S.; Dubois, J.-E. *Tetrahedron Lett.* **1972**, *13*, 165.

(8) (a) δ 2.23 (C-4 CH_3) in $CDCl_3$ for 4-methyl-19-norandrosta-1,3,5(10)-trien-17-one: ref 5b,c. (b) δ 2.16 (C-4 CH_3) in $CDCl_3$ for (20*R*)-4-methyl-19-norcholesta-1,3,5(10)-triene: Hussler, G.; Chappe, B.; Wehrung, P.; Albrecht, P. *Nature (London)* **1981**, *294*, 556 and ref 3b.

(9) (a) δ 2.25 (C-1 CH_3) in CCl_4 for (20*R*)-1-methyl-19-norcholesta-1,3,5(10)-triene: Dannenberg, H.; Gross, H. *Liebigs Ann. Chem.* **1966**, *692*, 180. (b) δ 2.29 (C-1 CH_3) in $CDCl_3$ for (20*R*)-1-methyl-19-norcholesta-1,3,5(10)-triene: Hussler, G. Ph.D. Thesis, Strasbourg, 1985.

(10) δ 2.75 (C-4 CH_3), 1.34 (C-17β CH_3) in $CDCl_3$ for (20*R*)-4,17β-dimethyl-18,19-dinorcholesta-1,3,5,7,9,11,13-heptaene: ref 3a.

(11) (a) δ 3.17 (C-1 CH_3) in $CDCl_3$ for (20*R*)-1,17β-dimethyl-18,19-dinorcholesta-1,3,5,7,9,11,13-heptaene: ref 3a. (b) δ 3.13 (C-1 CH_3) in $CDCl_3$ for (20*R*,24*R*)-1,17β-dimethyl-18,19-dinorstigmasta-1,3,5,7,9,11,13-heptaene: ref 3a. (c) δ 3.12 (C-1 CH_3) in CCl_4 for both 1,17β-dimethyl-18,19-dinorpregna-1,3,5,7,9,11,13-heptaene and 1,17β-dimethyl-18,19-dinorpregna-1,3,5,7,9,11,13,15-octaene: Riolo, J. Ph.D. Thesis, Strasbourg, 1985.

(12) (a) Yamada, T.; Morita, K. *Yukagaku* **1962**, *11*, 290 (*Chem. Abstr.* **1963**, *58*, 14058b). (b) Yamada, T.; Tsusumi, K. *Ibid.* **1964**, *13*, 469 (*Chem. Abstr.* **1965**, *63*, 18201g).

11,13-heptaene (1).[3] A mixture of 210 mg (0.54 mmol) of **9** and 124 mg (0.54 mmol, 1 equiv) of PtO_2 in 10 mL of 1:2 benzene–hexane was hydrogenated at 60 psi for 1.5–2 h. The solution was filtered and concentrated. The crude product was crystallized from EtOAc to give 65 mg of **1**. A second crystallization gave an additional 25 mg (total yield, 43%) of **1**: mp 109–110 °C; ^{13}C NMR ($CDCl_3$) δ 12.0 (C-19 CH_3), 15.4, 19.0, 20.0, 20.1, 23.1, 27.1, 28.5, 29.2, 29.5, 29.9, 34.0, 41.9, 45.8, 127.2, 128.3, 129.4, 130.4, 131.0, 134.9, 139.7, 149.4; exact mass spectrum calcd for $C_{29}H_{38}$ 386.2973, found 386.2976.

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Supplementary Material Available: ^1H and COSY NMR spectra for (20*R*,22*E*,24*S*)-stigmasta-2,4,6,22-tetraene (**6a**) and ^1H NMR data and assignments for **1**, **5**, **6a**, **7**, **8**, and **9** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.