Absolute Configuration of the Antiinflamatory Sponge Natural Product Contignasterol

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Chiral auxiliary NMR analysis with C-22 (*R*/*S*)-MPA and (*R*/*S*)-MTPA esters has been used to demonstrate that the absolute configuration in the side chain of the antiinflammatory sponge metabolite contignasterol (**1**) is 22*R*, 24*R*.

Contignasterol (**1**),[1] isolated from *Petrosia contignata* collected in Papua New Guinea, was the first reported example of an emerging family of marine sponge steroids that are characterized by having in common the rare cis C/D ring junction and 15-keto, 6-α-hydroxyl, and 7-*β*-hydroxyl functionalities. Other known members of this family include xestobergsterols A to C,[2,3] 14*β*-tamosterone sulfate,[4] haliclostanone sulfate,[5] and clathriol.[6] Contignasterol[7] and other members of this family of steroids[2,6,8] inhibit the anti-Ige stimulated release of histamine from sensitized rat mast cells in a dose-dependent manner. On the basis of this promising biological activity,[9] contignasterol was chosen as the lead structure for an analogue synthesis program that identified IPL576-092 as a novel antiasthma/antiinflammatory agent.[10] IPL576-092 has been advanced to phase II human clinical trials.

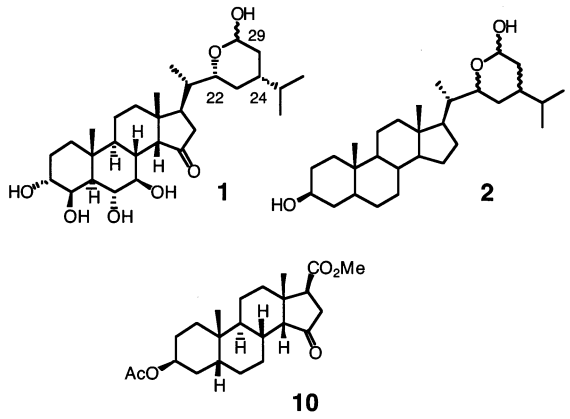
The initial structure elucidation of contignasterol (**1**) assigned only the relative stereochemistry at C-22 and C-24 in the steroid's side chain.[1] Subsequently, the synthesis of a series of model compounds (i.e., **2**) containing the four possible C-22/C-24 stereoisomers for the contignasterol side chain has been reported.[11] On the basis of comparisons of the proton NMR data obtained for these four stereoisomers with the NMR data reported for contignasterol (**1**), it was proposed that the side chain configuration in the natural product was (22*S*, 24*S*). To complete the structure elucidation of contignasterol (**1**), we have examined the absolute configuration of the side chain of the natural product. The details of this analysis, which has shown that contignasterol has the (22*R*, 24*R*) configuration, are reported below.

Contignasterol has a cyclic hemiacetal in its side chain, which exists in slow equilibrium with the open chain 22-hydroxy-29-aldehyde form. The secondary alcohol at C-22 appeared to be ideally suited for chiral auxiliary analysis of its absolute configuration using either MTPA or MPA esters.[12,13] A potential complicating factor in the analysis of the C-22 configuration was the polyhydroxyl nature of contignasterol, which we anticipated would result in the formation of multiple esters with the chiral reagent. To circumvent this complication, the hydroxyl groups on the nucleus were protected as acetate esters prior to selectively liberating the C-22 alcohol from the cyclic hemiacetal and forming the C-22 chiral auxiliary esters. This sequence of transformations was accomplished as shown in Figure 1.

Reaction of contignasterol with acetic anhydride in pyridine in the presence of a catalytic amount of (dimethylamino)pyridine gave pentaacetylcontignasterol (**3**), as a mixture of C-29 epimers, in good yield. Treatment of the pentaacetates with BF_3 etherate in aqueous acetonitrile selectively hydrolyzed the C-29 acetates to give the tetraacetate **4**. The C-22 alcohol could be liberated from the hemiacetal in **4** by trapping the C-29 aldehyde with 1,2-ethanedithiol to give the cyclic dithiane **5**. Reaction of **5** with the (*S*)- and (*R*)-MTPA acid chlorides gave the Mosher esters **6** and **7**, while treatment with (*R*)- and (*S*)-MPA and DCC gave the MPA esters **8** and **9**, respectively.

Recent work by Riguera's group has shown that MPA esters are preferable to MTPA esters for determining the absolute configuration of secondary alcohols.[12] Therefore, MPA was the preferred chiral auxiliary for determining the C-22 configuration of contignasterol. The decision to carry out the apparently redundant configurational analysis with MTPA was based on the report by Izzo et al.[11] that they had used (*R*) and (*S*) C-22 MTPA esters to verify the side chain configurations in the diastereomers of the model compound **2**. Using the same chiral auxiliary to make C-22 esters of contignasterol provided a more direct comparison of the C-22 configurations in the model compounds and contignasterol (**1**), and it provided independent confirmation of the C-22 configuration determined with the MPA esters (Figure 2).

Table 1 lists the ^{1}H chemical shifts for the esters **6**, **7**, **8**, and **9** and the $\Delta\delta^{RS}$ value for each proton in both the MTPA and MPA (*R*/*S*) pairs. Applying the empirical rules[12,13] to both sets of $\Delta\delta^{RS}$ values predicts that the C-22 configuration in contignasterol is (*R*), and therefore, the C-24 configuration is also (*R*).[1]



The CD spectrum of contignasterol (**1**) shows a negative Cotton effect at 300 nm attributed to the n to π^* transition of the C-15 ketone. Comparison of this CD data with that

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Table 1. NMR Data for (*R*),(*S*)-MPA Esters **8** and **9**, (*R*),(*S*)-MTPA Esters **6** and **7**, and Dithioacetal (**5**) Recorded at 400 MHz in CD_2Cl_2 at 300 K

	δ ^{1}H (*R*)-MPA (**8**)	δ ^{1}H (*S*)-MPA (**9**)	$\Delta\delta^a$	δ ^{1}H (*R*)-MTPA (**6**)	δ ^{1}H (*S*)-MTPA (**7**)	$\Delta\delta^b$	δ ^{1}H dithioacetal (**5**)	δ ^{13}C dithioacetal (**5**)
1								31.9
2				1.70, 1.83	1.70, 1.83	0.00, 0.00		21.9
3	4.80	4.81	−0.01	4.82	4.82	0.00	4.81	68.5
4	4.96	4.97	−0.01	4.98	4.98	0.00	4.97	67.3
5	1.83	1.86	−0.04	1.89	1.89	0.00	1.87	44.4
6	5.00	5.02	−0.02	5.03	5.04	0.01	5.02	70.7
7	5.88	5.92	−0.03	5.93	5.94	0.01	5.95	74.8
8	1.83	1.90	−0.08	1.94	1.94	0.00	1.87	36.9
9				1.23	1.22	−0.01		46.5
10								36.3
11								21.4
12								37.8
13								42.0
14	2.14	2.24	−0.10	2.27	2.25	−0.02	2.73	50.9
15								218.8
16α	2.18	2.42	−0.24	2.46	2.42	−0.04	2.41	39.2
16*β*	1.89	2.11	−0.22	2.24	2.21	−0.03	2.21	
17	1.20	1.62	−0.42	1.69	1.61	−0.08	1.92	46.5
18	1.05	1.16	−0.11				1.20	19.9
19	1.06	1.08	−0.02				1.09	14.2
20	1.60	1.94	−0.34	2.10	2.01	−0.09	1.84	41.1
21	0.82	1.01	−0.19	1.10	0.88	−0.22	0.93	17.9
22	5.12	5.08	0.04	5.26	5.20	−0.06	3.49	72.6
23	1.44, 1.82	1.32, 1.55	0.12, 0.27	1.35, 1.70	1.35, 1.67	0.00, −0.03	1.20, 1.59	37.2
24								41.4
25	1.79	1.59	0.20	1.77	1.84	0.07	1.76	29.7
26/ 27	0.88, 0.77	0.65, 0.63	0.23, 0.14	0.85, 0.73	0.90, 0.76	0.05, 0.03	0.90, 0.79	19.9, 17.5
28	1.57, 1.77	1.44, 1.68	0.13, 0.09	1.47, 1.74	1.53, 1.81	0.06, 0.07	1.64, 1.82	40.3
29	4.54	4.33	0.21	4.33	4.43	0.10	4.56	52.9
30/ 31	3.20−3.28	3.17−3.23	0.04	3.12−3.22	3.17−3.26	0.05	3.25−3.15	38.3, 38.6

[a] $\Delta\delta = \delta_{(R)-\mathrm{MPA}} - \delta_{(S)-\mathrm{MPA}}$. [b] $\Delta\delta = \delta_{(S)-\mathrm{MTPA}} - \delta_{(R)-\mathrm{MTPA}}$.

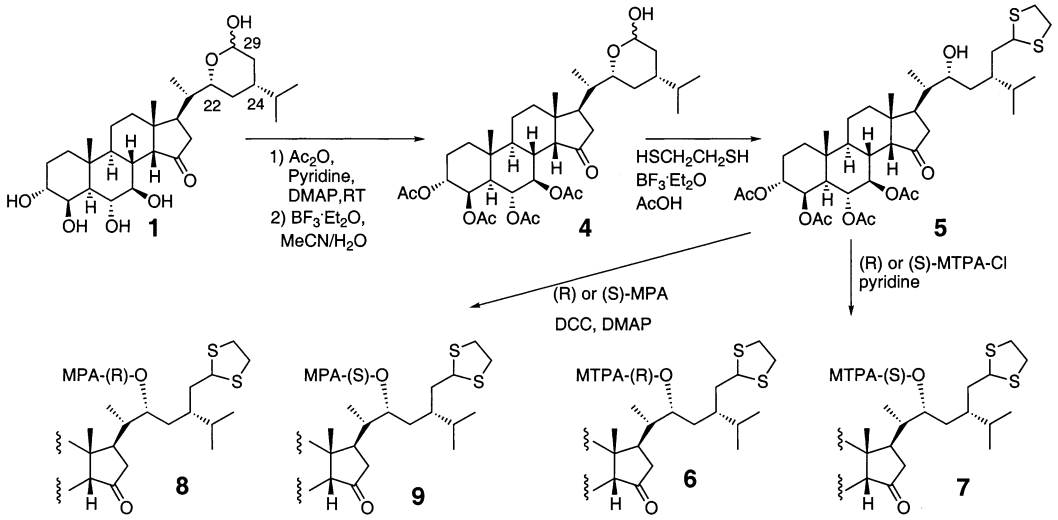

Figure 1. Chemical conversion of contignasterol (**1**) into the MTPA esters **6** and **7** and the MPA esters **8** and **9**.

for the model 15-keto-14*β* steroid **10**, which is reported to have a negative Cotton effect at 300 nm in its ORD curve,[14] confirmed that the nucleus of contignasterol has the standard steroidal configuration. The H-22 resonance (δ 3.33; benzene-d_6) of **1** showed a positive NOE when H-14 (δ 2.34) was irradiated in a difference NOE experiment,[1] confirming that the side chain was *β* at C-17. A coupling constant of 8.4 Hz was observed between H-20 (δ 1.84) and H-22 (δ 3.49) in the dithiane **5**, and there was no appreciable NOE observed between these resonances in a 1D NOESY experiment, suggesting that the protons were anti to each other. Strong NOEs observed between H-22 (δ 3.49) and both the H-16*β* (δ 2.21) and CH_3-21 (δ 0.94) resonances in 1D NOESY experiments carried out on **5** were consistent with the proposed anti orientation of H-20 and H-22 and the 22-*R* configuration shown in the Newman projections

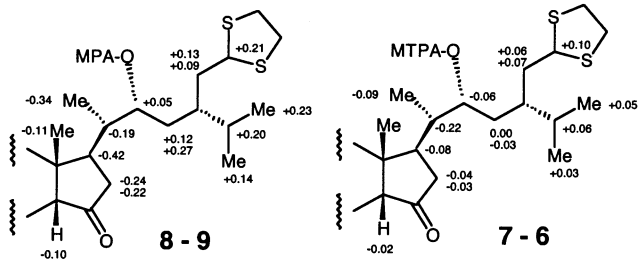

Figure 2. Selected $\Delta\delta$ ^{1}H NMR values in ppm for the MPA esters **8** and **9** and the MTPA esters **6** and **7**.

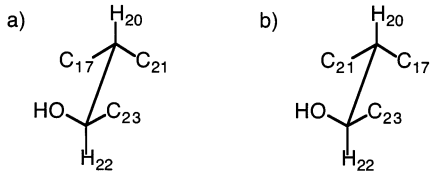

Figure 3. Newman projections for the C-20/C-22 bond in the dithiane **5** in which C-22 has the *R* configuration and H-20 and H-22 are anti.

in Figure 3a and b. Intense NOEs observed between the CH_3-21 (δ 0.94) and H-23 (δ 1.21)/H-23′ (δ 1.60) resonances in **5** demonstrated that C-20 has the *S* configuration shown in Figure 3a, which is the normal C-20 configuration for a steroid. Therefore, the complete absolute configuration of contignasterol is 3*R*, 4*R*, 5*R*, 6*R*, 7*R*, 8*R*, 9*S*, 10*R*, 13*R*, 14*R*, 17*R*, 20*S*, 22*R*, 24*R*, as shown in **1**.

The 22*R*, 24*R* configurations assigned to contignastrol (**1**) in the current study are antipodal to the 22*S*, 24*S* configurations proposed by Izzo et al. on the basis of proton chemical shift comparisons with all of the possible diastereomers of model compound **2**. This discrepancy presumably reflects the sensitivity of the side chain proton chemical shifts to through-space shielding and deshielding interactions with functional groups in the steroid nucleus of contignasterol (**1**). The unfunctionalized nucleus of the model compound **2** likely cannot accurately mimic these interactions, making a simple proton chemical shift comparison between the model compounds and contignasterol an unreliable predictor of the side chain stereochemistry in **1**.

Experimental Section

General Experimental Procedures. Circular dichroism spectra were recorded on a JASCO J-76 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, on Bruker AV400 and AM400 spectrometers. ^{1}H chemical shifts are referenced to the residual CH_2Cl_2-d_2 signal (δ 5.32 ppm), and ^{13}C chemical shifts are referenced to the CH_2Cl_2-d_2 solvent peak (δ 53.8 ppm). FABMS were recorded on a Kratos Concept II HQ mass spectrometer. Commercially obtained reagents were used without further purification. All reactions were monitored by TLC (silica gel, 60F-54, Merck). HPLC was performed on a Alltech column (250 × 4.6 mm, *n*-hexane/ethyl acetate, 1 mL/min) monitored by a RI detector.

Isolation of Contignasterol. Contignasterol (**1**) was isolated as a white solid from the extracts of the sponge *Petrosia contignata* Thiele using the same procedure as reported in the original paper.[1]

Preparation of Contignasterol Pentaacetates (3). Contignaterol (**1**) (32.8 mg) was dissolved in 2 mL of pyridine and 2 mL of Ac_2O. A few crystals of DMAP were added, and the resulting mixture was stirred at RT for 18 h. The reaction solvents were removed by evaporation under high vacuum, and purification of the products was carried out by normal-phase HPLC, eluting with *n*-hexane/EtOAc (4:3) to yield the C-29α (6 mg) and C-29β (16 mg) contignasterol pentaacetates (**3**) (total isolated yield was 47.5%). The spectroscopic data for the pentatacetates (**3**) were identical to the literature values.[1]

Contignasterol Tetraacetate (4). The pentaacetates (**3**) of contignaterol (either C-29α or C-29β) (21.2 mg, 0.0295 mmol) were dissolved in 0.8 mL of MeCN containing 7.3 μL of H_2O and treated with BF_3·OEt_2 (6 μL, 0.048 mmol) at 0 °C for 1.5 h. The reaction was quenched with saturated $NaHCO_3$ (1 mL) and extracted with EtOAc (3 × 10 mL), and the EtOAc was dried with $MgSO_4$. After evaporation of the solvent, 18.4 mg (92%) of contignasterol tetraacetate (**4**) was obtained as a white solid, which was used without further purification. The ^{1}H NMR spectrum of **4** was not assigned. It is very complex because **4** exists as a slowly equilibrating mixture of C-29 epimers. FABHRMS $[M + Na]^+$ *m/z* 699.3715 (calcd for $C_{37}H_{56}NaO_{11}$, 699.3720).

Dithiane 5. Contignasterol tetraacetate (**4**) (10 mg in 28.7 μL of AcOH) was treated with a solution of 1,2-ethanedithiol (9.6 μL) and BF_3·OEt_2 (1.2 μL), and the resulting mixture was stirred at room temperature overnight. Aqueous $NaHCO_3$ (1 mL of a 1 M solution) and 7 mL of CH_2Cl_2 were added, and the organic layer was separated, evaporated in vacuo, and subjected to normal-phase HPLC (eluent: EtOAc/hexane (1:2)) to give pure dithiane **5** (yield 7.8 mg, 70%): ^{1}HNMR and ^{13}CNMR, see Table 1; FABHRMS $[M + H]^+$ *m/z* 753.3701 (calcd for $C_{39}H_{61}O_{10}S_2$, 753.3706).

Preparation of the (*R*)- and (*S*)-MTPA Esters 6 and 7. (*R*)- or (*S*)-MTPA chloride (0.5 mL) and 1 mL of pyridine were added to the dithiane **5**, and the mixture was stirred at RT overnight. The pyridine was removed in vacuo, and the (*R*)- and (*S*)-MTPA esters were purified by normal-phase HPLC (eluent: hexane/EtOAc (7:3)).

(*R*)-MTPA ester 6: ^{1}HNMR, see Table 1; FABHRMS $[M + H]^+$ *m/z* 969.4103 (calcd for $C_{49}H_{68}F_3O_{12}S_2$, 969.4104).

(*S*)-MTPA ester 7: ^{1}HNMR, see Table 1; FABHRMS $[M^+ + H]^+$ *m/z* 969.4105 (calcd for $C_{49}H_{68}F_3O_{12}S_2$, 969.4104).

(*R*)- and (*S*)-MPA Esters 8 and 9. (*R*)- or (*S*)-MPA, DCC, and the dithiane **5** in the molar ratio of 5:7:1 were added into 1 mL of anhydrous CH_2Cl_2 along with a few crystals of DMAP. The mixture was stirred at RT for 10 h, then filtered to remove the dicyclohexylurea, and the (*R*)- and (*S*)-MPA esters were purified by normal-phase HPLC (eluent: hexane/EtOAc (2:1)).

(*R*)-MPA ester 8: ^{1}HNMR, see Table 1; FABHRMS $[M + H]^+$ *m/z* 901.4229 (calcd for $C_{48}H_{69}O_{12}S_2$, 901.4230).

(*S*)-MPA ester 9: ^{1}HNMR, see Table 1; FABHRMS $[M + H]^+$ *m/z* 901.4229 (calcd for $C_{48}H_{69}O_{12}S_2$, 901.4230).

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