Synthesis of Methyl (20*R*,22*E*)- and (20*S*,22*E*)-3-Oxochola-1,4,22-trien-24-oate

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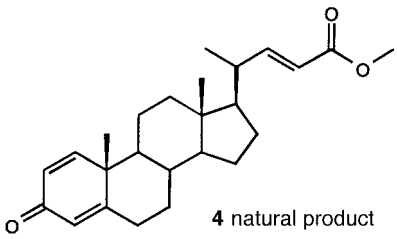
Dedicated to the memory of *V. Cerny*

Methyl (22*E*)-3-oxochola-1,4,22-trien-24-oate (**4**; $C_{25}H_{34}O_3$) is a naturally occurring steroid with unknown configuration at C(20). Starting from the (20*S*)-3-oxo-23,24-dinorchol-4-en-22-al (**1a**), we prepared both diastereoisomeric methyl esters **4a** and **4b** by a three-step procedure (*Scheme*). In the case of **4b**, the initial epimerization of aldehyde **1a** was followed by completion of the sequence and then separation *via* fractional crystallization to afford pure (20*R*)-methyl ester **4a** and its (20*S*)-diastereomer **4b**. Only the analytical data of the (20*S*)-compound **4b** were in good agreement with those reported for the natural product.

Introduction. – In pursuing their program designed for the discovery of new antifouling substances from marine benthic invertebrates, *Tomono et al.* [1] reported in 1999 the isolation of four unknown steroids from an octocoral *Dendronephthya sp.* of the order Alcyonacea. These compounds showed no antifouling activity against barnacle (*Balanus amphitrite*) larvae, but, instead, lethality to the barnacle larvae at a concentration of 100 μg/ml (LD_{100}). *Tomono et al.* identified one of the new compounds as methyl 3-oxochola-1,4,22-trien-24-oate (**4**) without specifying the configuration at C(20). No melting point was reported, but an optical rotation of $[\alpha]_D^{22} = +53.6$ ($CHCl_3$, $c = 0.28$) and detailed NMR data were given.

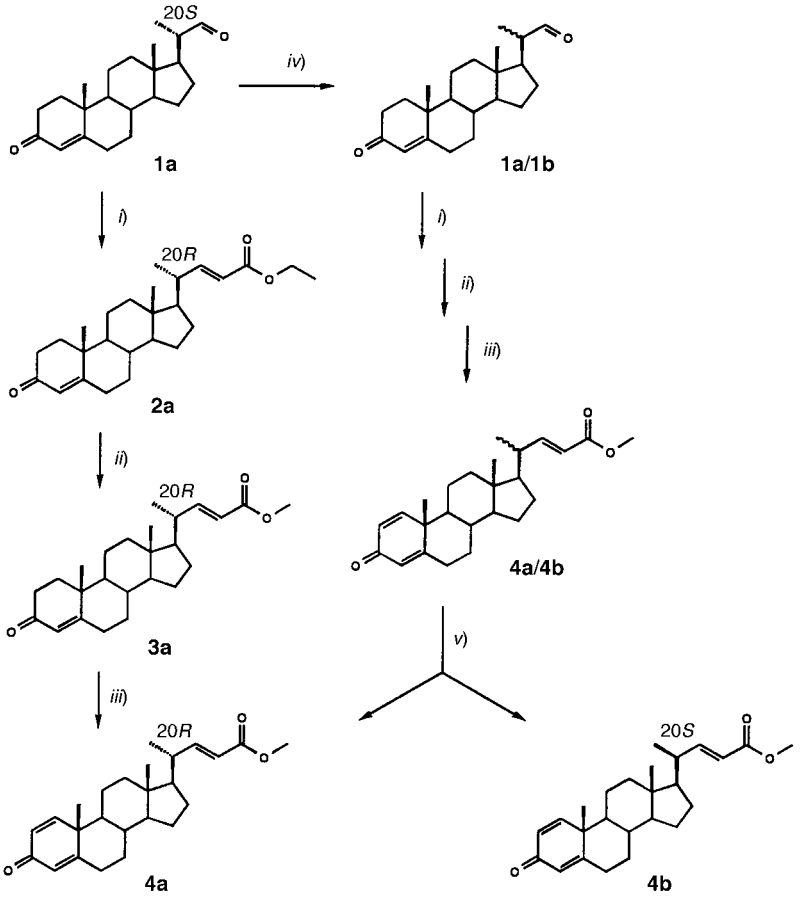


4 natural product

In this paper, we wish to report the preparation of both the (20*R*)- and (20*S*)-epimers **4a** and **4b**, their separation *via* fractional crystallization, their unambiguous proof of configuration by X-ray analysis, and the comparison of their spectral data with the naturally occurring steroid derivative.

Results and Discussion. – For the synthesis of the (20*R*)-methyl ester (**4a**), we started from (20*S*)-3-oxo-23,24-dinorchol-4-en-22-al (**1a**) (*Scheme*). *Horner-Wittig* reaction with (diethoxyphosphinyl)acetate [2] led to the corresponding ethyl ester **2a**,

Scheme. *Synthesis of (20*R,22E*)- and (20*S,22E*)-3-Oxochola-1,4,22-trien-24-oate* (**4a** and **4b**, resp.)



i) $(EtO)_2P(O)CH_2COOEt$, NaH, THF. *ii*) $TsOH \cdot H_2O$, MeOH. *iii*) DDQ, toluene. *iv*) H_2SO_4, H_2O, EtOH. *v*) Crystallization from cyclohexane.

and after transesterification in the presence of TsOH in MeOH, the methyl ester **3a** was obtained. Its corresponding acid had already been isolated from different marine organisms and characterized as **3a** [3][4]. Dehydration of **3a** by DDQ (=4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile) [5] finally gave methyl (20*R*,22*E*)-3-oxochola-1,4,22-trien-24-oate (**4a**). Appropriate crystals of **4a** were grown from cyclohexane, and its configuration (20*R*) has been determined by X-ray analysis [6].

In the *Table*, some relevant NMR chemical-shift data of **4a** are listed and compared to those of the natural product. As for the side-chain shift values, there are considerable and significant differences. Also the sign and value of the optical rotation of **4a** and of the natural product **4** are different [1]. So far, these observations indicate a configurational deviation in the natural product's side chain.

To get a reliable structure proof, the synthesis of the (20*S*)-methyl ester **4b** was undertaken. Since the acidity of H–C(20) of **4a** is low (no reaction with NaH at reflux

Table. *Comparison of the NMR Data of Both C(20)-Epimers* **4a** *and* **4b** *with Those Reported for the Natural Product* **4** [1]. δ in ppm.

		4b	Natural product **4**	**4a**
^{1}H-NMR:	Me(18)	0.66	0.69	0.73
	Me(19)	1.17	1.19	1.18
	Me(21)	0.94	0.96	1.03
	H–C(22)	6.81	6.82	6.77
	H–C(23)	5.74	5.75	5.70
	MeO	3.69	3.71	3.67
^{13}C-NMR:	C(12)	38.4	38.5	39.2
	C(13)	42.6	42.6	42.9
	C(14)	55.1	55.1	54.7
	C(16)	27.5	27.5	27.9
	C(17)	55.7	55.8	55.2
	C(20)	39.9	39.9	39.6
	C(21)	20.0	20.0	19.1
	C(22)	155.3	155.3	154.5
	C(24)	167.2	163.6	167.3

temperature), an epimerization of this compound is not suitable for this purpuse. Aldehyde **1a** should be a more promising candidate for inversion of configuration at C(20) by synthesis of the enolate or the corresponding enamine. After acid hydrolysis [7] of aldehyde **1a**, only a 1:2 mixture **1a**/**1b** ((*R*/*S*); determined by ^{1}H-NMR) could be obtained. The same ratio was determined after acid hydrolysis of the enamine 22-(piperidin-1-yl)-23,24-dinorchola-4,20(22)-dien-3-one, prepared from **1a** according to *Herr* and *Heyl* [7].

Since a chromatographic separation of the diastereoisomers **1a**/**1b** failed and crystallization resulted in enrichment of the starting (20*S*)-aldehyde **1a**, the epimer mixture **1a**/**1b** was used as starting material for the same sequence as described above (*Scheme*). Crystallization of the resulting methyl esters **4a**/**4b** from cyclohexane led to the pure (20*R*)-methyl ester **4a**. Then, after evaporation, the mother liquor consisted of the diastereomers **4b**/**4a** ((*S*/*R*)) in a ratio of *ca*. 5:1. Further crystallizations enriched the predominant epimer **4b**, leading to pure (20*S*)-methyl ester **4b**. The crystallizations were monitored by ^{1}H-NMR spectroscopy (*s* of MeO), as illustrated in the *Figure.*

The relevant NMR data of **4b** are very similar to those of natural product **4** (see *Table*), and the optical rotation measured for **4b** is of the same order as the one reported for **4** [1]. These data disclose that the (20*S*)-epimer is identical to the natural steroid (no m.p. is available for the natural product **4**).

To demonstrate the configuration at C(20), an X-ray-analysis of **4b** has been undertaken [8], too, which undoubtedly established its (20*S*) configuration. The tendency of the epimers to crystallize in different space groups (**4a**: $P2_1$, monoclinic; **4b**: $P2_12_12_1$, orthorhombic) might serve as an explanation for their simple separation *via* crystallization.

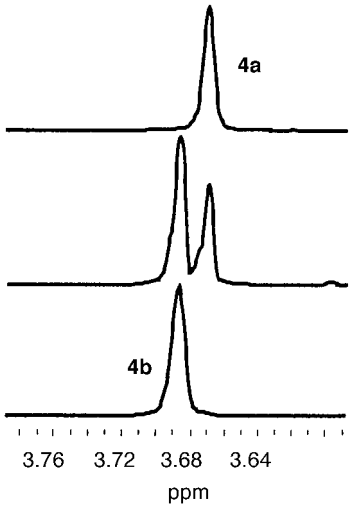


Figure. *Shift of the MeO* singlet *in the ^{1}H-NMR spectrum during crystallization of* **4a**/**4b**

Experimental Part

General. All reagents and solvents were commercially available and used as provided. Aldehyde **1a** was provided by *Schering Co*. THF and toluene were distilled from Na and stored over molecular sieves (4 Å), MeOH was distilled from Mg. All solns. were dried over anh. Na_2SO_4. TLC: *Merck* silica gel *60* F_{254} plates, detection with UV and phosphoromolybdic acid. Column chromatography (CC): silica gel *60* (230–400 mesh), *Merck*. M.p.: *Büchi 510*; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter; *c* in g/100 ml. IR Spectra: *Nicolet Impact-400-D* or *Perkin-Elmer 457* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX 400*; ^{1}H at 400.13 and ^{13}C at 100.6 MHz; δ in ppm rel. to internal standard Me_4Si (=0 ppm), *J* in Hz. MS: *Finnigan MAT-8230* or *TSQ-7000* (70 eV); in *m*/*z*.

*Ethyl (20*R,*22*E*)-3-Oxochola-4,22-dien-24-oate* (**2a**). A soln. of ethyl (diethoxyphosphinyl)acetate (10.31 g, 45 mmol) in dry THF (80 ml) is added dropwise at 0° to a stirred suspension of NaH (1.80 g, 45 mmol; 60% in mineral oil) in dry THF (150 ml). At the end of gas evolution, **1a** (9.85 g, 30 mmol) in dry THF (100 ml) is added dropwise, and the soln. is stirred overnight. After addition of H_2O (30 ml), the mixture is extracted with AcOEt (2 × 60 ml), the org. layer washed with sat. $NaHCO_3$ soln. (2 × 70 ml) and brine (70 ml), dried (Na_2SO_4), and evaporated, and the residue dried *in vacuo:* **2a** (11.85 g, 99%), sufficiently pure for the next step. R_f (cyclohexane/AcOEt 2:1) 0.41. M.p. 155–157°. $[\alpha]_D^{21} = +54.6$ ($CHCl_3$, $c = 0.28$). IR: 2940, 2908, 2819, 2850, 1237, 1721, 1676, 1652, 1614. ^{1}H-NMR (400.13 MHz, $CDCl_3$): 0.70 (*s*, Me(18)); 1.04 (*d*, $^3J = 6.8$, Me(21)); 1.13 (*s*, Me(19)); 1.23 (*t*, $^3J = 7.0$, $MeCH_2O$); 0.7–2.4 (*m*, 21 H); 4.12 (*q*, $^3J = 7.0$, $MeCH_2O$); 5.66 (*s*, H–C(4)); 5.68 (*d*, $^3J = 15.1$, H–C(23)); 6.77 (*dd*, $^3J = 8.8, 15.6$, H–C(22)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 12.1 (*q*, C(18)); 14.2 (*q*, $MeCH_2O$); 17.3 (*q*, C(19)); 20.9 (*t*, C(11)); 24.1 (*t*, C(15)); 28.0 (*t*, C(16)); 31.8 (*t*, C(1)); 32.8 (*t*, C(6)); 33.9 (*t*, C(7)); 35.5 (*d*, C(8)); 35.6 (*t*, C(2)); 38.5 (*s*, C(10)); 39.3 (*t*, C(12)); 39.6 (*d*, C(20)); 42.6 (*s*, C(13)); 53.6 (*d*, C(9)); 54.7 (*d*, C(14)); 55.6 (*d*, C(17)); 60.0 (*t*, $MeCH_2O$); 119.0 (*d*, C(23)); 123.7 (*d*, C(4)); 154.3 (*d*, C(22)); 166.9 (*s*, C(24)); 171.3 (*s*, C(5)); 199.4 (*s*, C(3)). MS: 398.

*Methyl (20*R,*22*E*)-3-Oxochola-4,22-dien-24-oate* (**3a**). A soln. of **2a** (11.96 g, 30 mmol) and $TsOH \cdot H_2O$ (1.14 g, 6 mmol) in dry MeOH (300 ml) is heated under reflux for 2 days. The MeOH is evaporated and the residue treated with AcOEt (250 ml) and H_2O (100 ml). The combined org. extract is washed with sat. $NaHCO_3$ (3 × 100 ml), H_2O (100 ml), and brine (100 ml), dried, and evaporated: **3a** (11.46 g, *ca*. 100%), sufficiently pure for the next step. Purification is possible by CC (cyclohexane/AcOEt 2:1, R_f 0.38). M.p. 146.5–148.5°. $[\alpha]_D^{21} = +54.5$ ($CHCl_3$, $c = 0.28$). IR: 2945, 2921, 2908, 2890, 2869, 2851, 1731, 1672, 1654, 1612, 1236. ^{1}H-NMR (400.13 MHz, $CDCl_3$): 0.71 (*s*, Me(18)); 0.87–1.24 (*m*, 7 H); 1.05 (*d*, $^3J = 6.5$, Me(21)); 1.15 (*s*, Me(19)); 1.38–2.43 (*m*, 14 H); 3.68 (*s*, MeO); 5.69 (*s*, H–C(4)); 5.71 (*d*, $^3J = 15.6$, H–C(23)); 6.79 (*dd*, $^3J = 9.0$, 15.6, H–C(22)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 12.1 (*q*, C(18)); 17.3 (*q*, C(19)); 19.1 (*q*, C(21)); 20.9 (*t*, C(11));

24.1 (*t*, C(15)); 28.0 (*t*, C(16)); 31.8 (*t*, C(1)); 32.8 (*t*, C(6)); 33.9 (*t*, C(7)); 35.5 (*d*, C(8)); 35.6 (*t*, C(2)); 38.5 (*s*, C(10)); 39.3 (*t*, C(12)); 39.6 (*d*, C(20)); 42.6 (*s*, C(13)); 51.3 (*q*, MeO); 53.6 (*d*, C(9)); 54.7 (*d*, C(14)); 55.5 (*d*, C(17)); 118.6 (*d*, C(23)); 123.7 (*d*, C(4)); 154.6 (*d*, C(22)); 167.3 (*s*, C(24)); 171.2 (*s*, C(5)); 199.3 (*s*, C(3)). MS: 385.0 ($[M+1]^+$).

*Methyl (20*R*,22*E*)-3-Oxochola-1,4,22-trien-24-oate* (**4a**). A soln. of **3a** (6.0 g, 15.6 mmol) and DDQ (4.53 g, 20.0 mmol) in dry toluene (175 ml) is heated under reflux for 24 h. After cooling to r.t. and filtration, the mixture is washed several times with 1% (*w*/*w*) KOH soln. (50 ml), H_2O (3 × 50 ml), and brine (2 × 50 ml), dried, and evaporated. Purification by CC (cyclohexane/AcOEt 2:1, R_f 0.30) yields **4a** (3.82 g, 64%) as a light yellow solid. After crystallization from cyclohexane, with addition of active charcoal, crystals suitable for X-ray-analysis are obtained. M.p. 167.5–168.5°. $[\alpha]_D^{21} = -16.8$ ($CHCl_3$, $c = 0.279$). ^{1}H-NMR (400.13 MHz, $CDCl_3$): 0.73 (*s*, Me(18)); 1.03 (*d*, $^3J = 6.5$, Me(21)); 1.18 (*s*, Me(19)); 0.98–1.25 (*m*, 7 H); 1.53–2.47 (*m*, 10 H); 3.67 (*s*, MeO); 5.70 (*d*, $^3J = 15.8$, H–C(23)); 6.02 (*s*, H–C(4)); 6.18 (*d*, $^3J = 10.0$, H–C(2)); 6.77 (*dd*, $^3J = 9.0$, 15.8, H–C(22)); 7.00 (*d*, $^3J = 10.0$, H–C(1)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 12.2 (*q*, C(18)); 18.6 (*q*, C(19)); 19.1 (*q*, C(21)); 22.7 (*t*, C(11)); 24.3 (*t*, C(15)); 27.9 (*t*, C(16)); 32.8 (*t*, C(6)); 33.5 (*t*, C(7)); 35.4 (*d*, C(8)); 39.2 (*t*, C(12)); 39.6 (*d*, C(20)); 42.9 (*s*, C(13)); 43.5 (*s*, C(10)); 51.3 (*q*, MeO); 52.2 (*d*, C(9)); 54.7 (*d*, C(14)); 55.2 (*d*, C(17)); 118.7 (*d*, C(23)); 123.8 (*d*, C(4)); 127.4 (*d*, C(2)); 154.5 (*d*, C(22)); 155.8 (*d*, C(1)); 167.3 (*s*, C(24)); 169.2 (*s*, C(5)); 186.3 (*s*, C(3)). MS: 383.0 ($[M+1]^+$).

3-Oxo-23,24-dinorchol-4-en-22-al (**1a**/**1b**). (20*S*)-Aldehyde **1a** (6.57 g, 20 mmol) is dissolved in a mixture of EtOH (320 ml), conc. H_2SO_4 soln. (64 ml), and H_2O (64 ml) and heated under reflux for 20 min. The mixture is poured onto ice (400 g) and extracted with Et_2O (1 × 350 ml, 2 × 150 ml). The combined org. extract is washed with H_2O (3 × 150 ml) and brine (150 ml), dried, and evaporated: **1a**/**1b** ((*S*/*R*)) *ca.* 1:2. Colorless solid. M.p. 124–126°. R_f (cyclohexane/AcOEt 2:1) 0.36. $[\alpha]_D^{21} = +94.8$ ($CHCl_3$, $c = 0.28$). IR: 2934, 1727, 1668. ^{1}H-NMR (400.13 MHz, $CDCl_3$)[1]): 0.66 (*s*, 4 H; *Me(18)); 0.71 (*s*, 2 H, Me(18)); 0.94 (*d*, $^3J = 6.8$, 4 H, *Me(21)); 1.07 (*d*, $^3J = 6.8$, 2 H, Me(21)); 1.11 (*s*, 4, *Me(19)); 1.13 (*s*, 2 H, Me(19)); 0.66–1.23 (*m*, 4 H); 1.26–2.45 (*m*, 18 H); 5.67 (*s*, 2 H, H–*C(4), H–C(4)); 9.47 (*d*, $^3J = 5.0$, 1.3 H, H–*C(22)); 9.51 (*d*, $^3J = 3.5$, 0.7 H, H–C(22)). ^{13}C-NMR (100.6 MHz, $CDCl_3$)[1]): 12.2 (*q*, C(18)); 12.8 (*q*, *C(18)); 13.3 (*q*, C(21)); 13.5 (*q*, *C(21)); 17.3 (*q*, (C(19), *C(19)); 20.6 (*t*, *C(11)); 20.8 (*t*, C(11)); 23.7 (*t*, *C(15)); 24.4 (*t*, C(15)); 26.3 (*t*, *C(16)); 26.9 (*t*, C(16)); 31.8 (*t*, C(1), *C(1)); 32.7 (*t*, C(6), *C(6)); 33.8 (*t*, C(7), *C(7)); 35.4 (*d*, *C(8)); 35.5 (*d*, C(8)); 35.6 (*t*, C(2), *C(2)); 38.1 (*s*, *C(10)); 38.5 (*s*, C(10)); 39.2 (*t*, C(12), *C(12)); 42.1 (*s*, *C(13)); 42.9 (*s*, C(13)); 48.7 (*d*, *C(17)); 49.3 (*d*, C(17)); 50.8 (*d*, C(20)); 51.6 (*d*, *C(20)); 53.6 (*d*, C(9), *C(9)); 55.0 (*d*, C(14)); 55.2 (*d*, *C(14)); 123.8 (*d*, C(4), *C(4)); 171.1 (*s*, C(5), *C(5)); 199.5 (*s*, C(3)); 199.5 (*s*, *C(3)); 204.8 (*d*, C(22)); 205.6 (*d*, *C(22)). MS: 328.8 ($[M+1]^+$).

*Methyl (20*S*,22*E*)-3-Oxochola-1,4,22-trien-24-oate* (**4b**). As described for (20*R*)-epimer **4a**, starting from **1a**/**1b**. The crude product **4a**/**4b** is purified by CC (cyclohexane/AcOEt 2:1; R_f 0.30). Fractional crystallization from cyclohexane leads to pure (20*R*)-methyl ester **4a**. The mother liquor is evaporated, and after 4 crystallizations from cyclohexane (once with addition of active charcoal), pure (20*S*)-methyl ester **4b** is obtained after standing overnight at r.t. Fine needles. M.p. 138–139°. These crystals are suitable for X-ray analysis. $[\alpha]_D^{21} = +90.7$ ($CHCl_3$, $c = 0.279$). IR: 2972, 2942, 2907, 2887, 2868, 2849, 1730, 1667, 1654, 1623, 1601, 1268, 1235. ^{1}H-NMR (400.13 MHz, $CDCl_3$): 0.66 (*s*, Me(18)); 0.94 (*d*, $^3J = 6.5$, Me(21)); 1.17 (*s*, Me(19)); 0.93–1.25 (*m*, 6 H); 1.50–2.46 (*m*, 11 H); 3.69 (*s*, MeO); 5.74 (*d*, $^3J = 15.5$, H–C(23)); 6.02 (*s*, H–C(4)); 6.17 (*d*, $^3J = 10.0$, H–C(2)); 6.81 (*dd*, $^3J = 10.0$, 15.5, H–C(22)); 6.98 (*d*, $^3J = 10.0$, H–C(1)). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 12.3 (*q*, C(18)); 18.6 (*q*, C(19)); 20.0 (*q*, C(21)); 22.5 (*t*, C(11)); 24.1 (*t*, C(15)); 27.5 (*t*, C(16)); 32.8 (*t*, C(6)); 33.5 (*t*, C(7)); 35.4 (*d*, C(8)); 38.4 (*t*, C(12)); 39.9 (*d*, C(20)); 42.6 (*s*, C(13)); 43.5 (*s*, C(10)); 51.4 (*q*, MeO); 52.3 (*d*, C(9)); 55.1 (*d*, C(14)); 55.7 (*d*, C(17)); 118.7 (*d*, C(23)); 123.8 (*d*, C(4)); 127.4 (*d*, C(2)); 155.3 (*d*, C(22)); 155.9 (*d*, C(1)); 167.2 (*s*, C(24)); 169.2 (*s*, C(5)); 186.4 (*s*, C(3)). MS: 383.0 ($[M+1]^+$).

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