Syntheses of 7-Alkyl-1,3,6-trimethylpyrrolo[2,3-*d*]pyrimidines and 4-Alkylamino-2,5-dimethyl-2,3-dihydrofuro[3,2-*e*]pyrimidines

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7-Alkyl-1,3,6-trimethylpyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones were readily synthesized by treatment of 6-alkylamino-5-allyl-1,3-dimethyluracils, which were derived from 5-allyl-6-chloro-1,3-dimethyluracil and alkylamines, with bis(acetonitrile) palladium (II) chloride. In addition, 4-alkylamino-2,5-dimethyl-2,3-dihydrofuro[3,2-*e*]pyrimidin-6-ones were easily synthesized by treatment of 5-allyl-6-chloro-1-methyluracil with alkylamines.

Keywords pyrrolo[2,3-*d*]pyrimidine; 5-allylbarbituric acid; 5-allyl-6-chlorouracil; palladium-catalyzed cyclization; $PdCl_2$-$(CH_3CN)_2$; furo[3,2-*e*]pyrimidine

6-Functionalized 5-allylpyrimidines can be used as starting materials for the synthesis of condensed pyrimidine rings.[2)] Some of which have interesting biological activities. For example, 6-chlorouracils containing allyl and alkyl groups display virostatic and cytostatic activities.[3)] Our interest in the 1,3-dialkyl-5-allyl-6-chlorouracils as potential bioactive substances and as starting materials for the syntheses of pyrrolo[2,3-*d*]pyrimidines, which can be regarded as analogues of antitumor antibiotics, tubercidin (**1**),[4)] sangivamycin (**2**),[5)] and toyocamycin (**3**),[6)] prompted us to investigate possible pathways for the syntheses of condensed nitrogeneous heterocycles.

5-Allylbarbituric acid (**4**) has been synthesized by cyclization reaction between urea and ethyl allylmalonate in 37% yield.[7)] We could raise the yield of **4** to 60% by the use of equimolar dry sodium methoxide (instead of 2 molar eq of ethanolic sodium ethoxide) in acetone. 1-Methyl- or 1,3-dimethylurea, however, failed to react with the above ester under similar conditions. Because *N*-unsubstituted 6-chlorouracil has usually been obtained by partial hydrolysis of 2,4,6-trichloropyrimidine,[8)] we attempted the synthesis of 5-allyl-2,4,6-trichloropyrimidine (**5**)[9)] by chlorination of **4** with $POCl_3$ in the presence of *N*,*N*-diethylaniline. It turned out that this reaction afforded only **5** in 50% yield. Alkaline hydrolysis of **5** with 4 molar eq excess of NaOH in water gave 41% yield of 5-allyl-6-chlorouracil (**6**). In the case of added water (0.56 mol), the reaction of **4** (0.10 mol) with $POCl_3$ (1.07 mol) and *N*,*N*-dimethylaniline (0.08 mol) proceeded smoothly, giving rise to **6** as the sole isolable product in 54% yield. Under the above conditions, pyrophosphoryl chloride that was formed by the reaction of $POCl_3$ and water[10)] may be the actual chlorinating reagent. The structure of **6** was supposed to be 5-allyl-6-chlorouracil because a series of reactions starting with **6** gave rise to **11a** *via*. **8**. Spectral data of **6** are also consistent with the assigned structure (*vide infra*).

Alkylation of **6** with an excess of MeI in the presence of K_2CO_3 in dimethylsulfoxide (DMSO) at 70 °C gave rise to 5-allyl-6-chloro-1,3-dimethyluracil (**7**) in 75% yield. When an equimolar amount of MeI was used under similar conditions, the yield of 5-allyl-6-chloro-1-methyluracil (**8**)

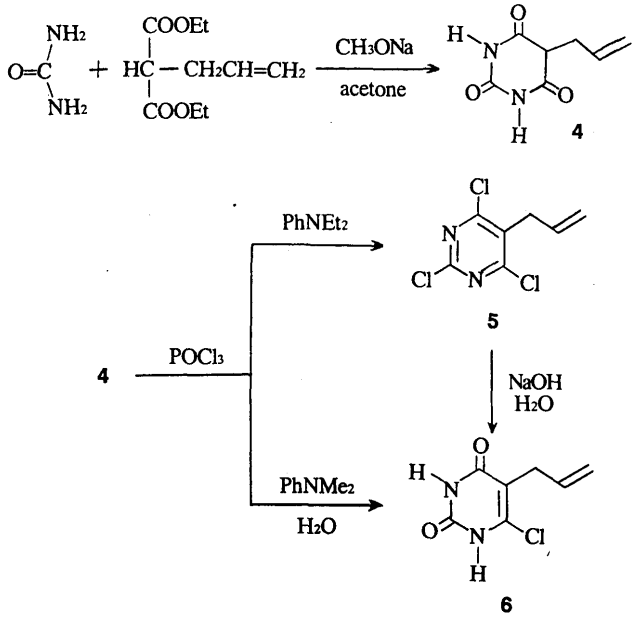


Chart 1

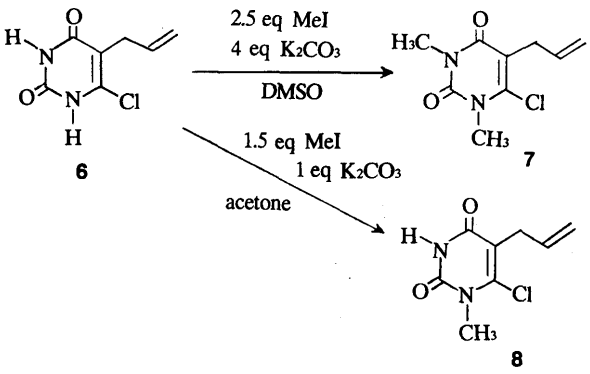
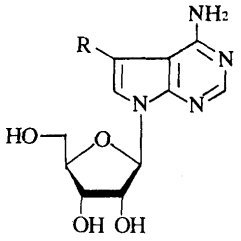
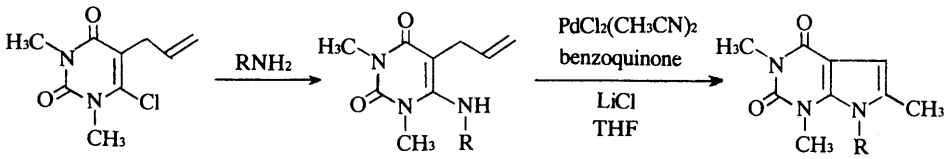


Chart 2



1 : tubercidin R=H
2 : sangivamycin R=$CONH_2$
3 : toyocamycin R=CN

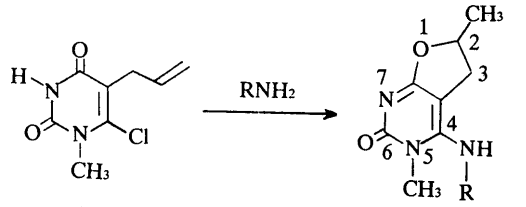
Fig. 1



7 9 a–f 10 a–f

a: R=CH_3 **d**: R=CH_2CH_2OH
b: R=C_2H_5 **e**: R=$CH_2C_6H_5$
c: R=C_3H_7 **f**: R=H

Chart 3



8 11

a: R=$CH_2C_6H_5$
b: R=CH_3
c: R=H

Chart 4

was 20%. A satisfactory result (71% yield of **8**) was obtained when MeI and K_2CO_3 were used in a molar ratio of 1.5 to 0.5 in acetone instead of DMSO. The structure of **8** was supported by its ultraviolet (UV) spectra in alkaline media. As is well established,[11] 1- and 3-alkyluracil each have characteristic UV spectra, the latter absorbing at longer wavelength. The UV spectrum of **6** was quite similar to that of 1-alkyluracil and not to that of 3-alkyluracil. Treatment of **7** with several alkylamines furnished the required 6-aminouracils **9a**—**f** in good yields.

Hegedus and co-workers[12] have prepared indole derivatives from 2-allylanilines by using a palladium catalyst. For our cyclization (from **9** and **10**) we applied their method of palladium-catalyzed cyclization to obtain pyrrolo[2,3-*d*]pyrimidines. Thus, the 6-aminouracils **9a**—**e** were treated with 1 molar eq of benzoquinone and 10 molar eq of LiCl in tetrahydrofuran (THF) in the presence of a catalytic amount of $PdCl_2(CH_3CN)_2$, at room temperature to give the required 7-alkylpyrrolo[2,3-*d*]pyrimidines **10a**—**e** in poor or moderate yields. In the case of unsubstituted 6-aminouracil **9f**, this reaction proceeded at 40—50 °C under palladium-catalyzed conditions and gave the pyrrolo[2,3-*d*]pyrimidine derivative **10f** in 55% yield.

On the other hand, treatment of **8** with alkylamines gave the 2,3-dihydrofuro[3,2-*e*]pyrimidines **11a**—**c** in moderate yields. The mass spectra (MS) and proton nuclear magnetic resonance (^{1}H-NMR) spectra of these compounds are consistent with those of the assigned bicyclic structure. In order to confirm the structure, we carried out X-ray crystal structure analysis of **11a**.

The structures of other newly synthesized compounds were confirmed by MS, high-resolution mass spectra (High MS), ^{1}H-NMR, and elemental analysis.

The results may be summarized as follows. i) The modified conditions allowed us to obtain 5-allylbarbituric acid in high yields. ii) Chlorination of 5-allylbaribituric acid was investigated and a procedure was developed to obtain 5-allyl-6-chlorouracil directly from 5-allylbarbituric acid. iii) Methylation of 5-allyl-6-chlorouracil was studied. iv) A synthetic procedure for pyrrolo[2,3-*d*]pyrimidines was developed. v) 2,3-Dihydrofuro[3,2-*e*]pyrimidines were prepared easily from 5-allyl-6-chloro-1-methyluracil with alkylamines, and the structure of the key compound (**11a**) was confirmed by means of X-ray analysis.

Experimental

General Melting points were determined in a capillary tube and are uncorrected. MS and High MS were recorded on JMS-DX 300 and JMA 3500 instruments. ^{1}H-NMR spectra were recorded on a Varian VXR-300 spectrometer in $CDCl_3$ or in DMSO-d_6. Chemical shifts are expressed in terms of δ values. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; qu, quintet; se, sextet; m, multiplet; br, broad. Microanalyses were performed by the staff in the Microanalytical Laboratory of this school. Thin layer chromatography (TLC) was performed on Kieselgel 60 GF_{254} (Merck) and spots were detected under UV light. Unless otherwise stated, the solvents were removed with a rotary evaporator and a water aspirator (*ca.* 20 mmHg).

5-Allylbarbituric Acid (4) A mixture of dry sodium methoxide [prepared from sodium (5.75 g, 0.25 atom) and methanol (150 ml)], urea (15 g, 0.25 mol), diethyl allylamlonate (50 g, 0.25 mol), and acetone (50 ml) was stirred under reflux for 7 h. The precipitate was collected by filtration, washed with acetone, suspended in water (100 ml), and acidified with concentrated aqueous HCl to pH 1—2. The precipitate was filtered off and recrystallized from EtOH to give 25 g (60%) of **4** as colorless needles, mp 170 °C (lit.[7] mp 167 °C). MS *m/z*: 168 (M^+). *Anal.* Calcd for $C_7H_8N_2O_3$: C, 50.00; H, 4.80; N, 16.66. Found: C, 49.87; H, 4.95; N, 16.51.

5-Allyl-2,4,6-trichloropyrimidine (5) A mixture of **4** (16.8 g, 0.1 mol), *N,N*-diethylaniline (10 ml), and $POCl_3$ (75 ml) was boiled for 3 h. The excess $POCl_3$ was removed under reduced pressure, and the residue was poured onto ice (200 g), extracted with $CHCl_3$ (100 ml × 3), and dried over Na_2SO_4. The solvent was removed and the residue was distilled at 120—122 °C/5 mmHg to give **5** (11.2 g, 50%) as a colorless oil, which spontaneously crystallized on standing, mp 38—40 °C (lit.[9] mp 39 °C). MS *m/z*: 222, 224, 226, and 228 (M^+). *Anal.* Calcd for $C_7H_5Cl_3N_2$: C, 37.62; H, 2.25; N, 12.57; Cl, 47.59. Found: C, 37.48; H, 2.39; N, 12.36; Cl, 47.71.

5-Allyl-6-chlorouracil (6) i) A mixture of **5** (4.5 g, 0.02 mol), NaOH (3.2 g, 0.08 mol), and water (50 ml) was boiled with stirring for 10—12 h, until a clear solution was obtained. After cooling, the solution was washed with ether (50 ml) and the water phase was acidified with concentrated aqueous HCl to pH 3—4. The crystals formed after standing for 12 h at 10 °C were collected by filtration, and recrystallized from EtOH to give **6** (1.5 g, 41%) as colorless needles, mp 214—216 °C. ii) $POCl_3$ (100 ml) added dropwise to a mixture of **4** (16.8 g, 0.1 mol), *N,N*-dimethylaniline (10 ml), and water (10 ml) during 1 h. The mixture was boiled for 45 min, the excess $POCl_3$ was removed *in vacuo*, and the residue was poured onto ice (270 g). After cooling, the precipitate was collected by filtration, washed with ether (100 ml), and dried at 100 °C to give **6** (10.1 g, 54%) as colorless needles, mp 215—217 °C (EtOH). MS *m/z*: 186 and 188 (M^+). UV λ_{max}^{EtOH} nm (log ε): 262 (3.67). UV $\lambda_{max}^{0.1\,N\,aq.NaOH-EtOH(1:10)}$ nm (log ε): 290 (3.98). ^{1}H-NMR (DMSO-d_6) δ: 3.00—3.07 (2H, br, $-CH_2CH=CH_2$), 4.97 (1H, d, $-CH_2CH=CH_2$, J=4.0 Hz), 5.00 (1H, d, $-CH_2CH=CH_2$, J=5.8 Hz), 5.74 (1H, ddt, $-CH_2CH=CH_2$, J=2.5, 4.0, 5.8 Hz), 11.35 (1H, br s, NH),

11.90 (1H, br, NH). *Anal.* Calcd for $C_7H_7ClN_2O_2$: C, 45.16; H, 3.76; N, 15.05; Cl, 19.09. Found: C, 44.91; H, 3.80; N, 14.93; Cl, 18.87.

5-Allyl-6-chloro-1,3-dimethyluracil (7) A mixture of **6** (9.3 g, 50 mmol), K_2CO_3 (13.8 g, 0.1 mol), and MeI (17.7 g, 0.125 mol) in DMSO (50 ml) was stirred at 60—70 °C for 1 h, then cooled. A 4% (w/v) aqueous solution (100 ml) of NaOH was added, and the mixture was extracted with ether (50 ml × 3). The organic phase was dried over Na_2SO_4, the solvent was removed, and the residue was distilled at 120—130 °C/3 mmHg to give **7** (8.0 g, 75%) as an oil, which crystallized on standing, mp 38—40 °C. High MS Calcd for $C_9H_{11}ClN_2O_2$: 214.051, 216.048. Found: 214.049, 216.048. ^{1}H-NMR ($CDCl_3$) δ: 3.24 (2H, dt, $-C\underline{H}_2CH=CH_2$, J=1.5, 6.0 Hz), 3.32, 3.55 (3H each, s, NCH_3), 5.01 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.5, 10.0 Hz), 5.07 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.5, 17.0 Hz), 5.77 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=6.0, 10.0, 17.0 Hz).

5-Allyl-6-chloro-1-methyluracil (8) A mixture of **6** (3.72 g, 20 mmol), K_2CO_3 (1.38 g, 10 mmol), and MeI (4.26 g, 30 mmol) in acetone (100 ml) was stirred with boiling for 10 h. The mixture was filtered, and the filtrate was evaporated to dryness. The residue was recrystallized from AcOEt to give **8** (2.83 g, 71%) as colorless needles, mp 186—188 °C. MS m/z: 200 and 202 (M^+). UV λ_{max}^{EtOH} nm (log ε): 267 (4.05). UV $\lambda_{max}^{0.1\,N\,aq.NaOH-EtOH(1:10)}$ nm (log ε): 266 (3.91). ^{1}H-NMR ($CDCl_3$) δ: 3.25 (2H, dt, $-C\underline{H}_2CH=CH_2$, J=1.5, 6.0 Hz), 3.45 (3H, s, NCH_3), 5.03 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 10.0 Hz), 5.10 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.5, 17.0 Hz), 5.79 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=6.0, 10.0, 17.0 Hz), 9.96 (1H, br, NH). *Anal.* Calcd for $C_8H_9ClN_2O_2$: C, 47.89; H, 4.52; N, 14.46; Cl, 17.67. Found: C, 47.90; H, 4.65; N, 14.24; Cl, 17.52.

5-Allyl-1,3-dimethyl-6-methylaminouracil (9a) A mixture of **7** (2.15 g, 10 mmol), 40% aqueous solution of methylamine (4 ml), and water (10 ml) was refluxed for 1 h, then extracted with $CHCl_3$ (15 ml × 2). The organic phase was dried over Na_2SO_4, the solvent was removed, and the residue was recrystallized from ether to give **9a** (1.6 g, 76%) as colorless needles, mp 73—75 °C. MS m/z: 209 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.79 (3H, d, $NHC\underline{H}_3$, J=5.5 Hz), 3.20 (2H, d, $-C\underline{H}_2CH=CH_2$, J=5.5 Hz), 3.30, 3.40 (3H each, s, NCH_3), 3.94 (1H, br q, $N\underline{H}CH_3$, J=5.5 Hz), 5.03 (1H, br d, $-CH_2CH=C\underline{H}_2$, J=9.5 Hz), 5.04 (1H, br d, $-CH_2CH=C\underline{H}_2$, J=17.0 Hz), 5.81 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=5.5, 9.5, 17.0 Hz). *Anal.* Calcd for $C_{10}H_{15}N_3O_2$: C, 57.40; H, 7.22; N, 20.08. Found: C, 57.16; H, 7.19; N, 19.98.

5-Allyl-6-ethylamino-1,3-dimethyluracil (9b) A mixture of **7** (1.50 g, 7 mmol) and 70% aqueous solution of ethylamine (3.5 ml) was refluxed for 2 h. The reaction mixture was worked up in a manner similar to that described above for **9a**, giving **9b** (1.4 g, 89%) as colorless needles, mp 99—101 °C. MS m/z: 223 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, $CH_2C\underline{H}_3$, J=7.0 Hz), 3.06 (2H, dq, $-NHC\underline{H}_2CH_3$, J=7.0, 7.0 Hz), 3.21 (2H, dt, $-C\underline{H}_2CH=CH_2$, J=1.5, 6.0 Hz), 3.32, 3.40 (3H each, s, NCH_3), 3.70 (1H, br t, $-N\underline{H}CH_2-$, J=7.0 Hz), 5.04 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.2, 1.5, 10.0 Hz), 5.06 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.2, 1.5, 17.0 Hz), 5.82 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=6.0, 10.0, 17.0 Hz). *Anal.* Calcd for $C_{11}H_{17}N_3O_2$: C, 59.17; H, 7.68; N, 18.82. Found: C, 58.98; H, 7.72; N, 18.71.

5-Allyl-1,3-dimethyl-6-propylaminouracil (9c) A mixture of **7** (1.5 g, 7 mmol) and propylamine (3.5 ml) was refluxed for 2 h. The reaction mixture was worked up in a manner similar to that described above for **9a** to give **9c** (1.3 g, 81%) as colorless needles, mp 103—105 °C. MS m/z: 237 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, $-CH_2CH_2C\underline{H}_3$, J=7.0 Hz), 1.60 (2H, m, $-CH_2C\underline{H}_2CH_3$), 2.98 (2H, q, $-NHC\underline{H}_2CH_2-$, J=7.0 Hz), 3.23 (2H, dt, $-C\underline{H}_2CH=CH_2$, J=1.5, 6.0 Hz), 3.33, 3.41 (3H each, s, NCH_3), 3.78 (1H, br t, $-N\underline{H}CH_2CH_2-$, J=7.0 Hz), 5.05 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.5, 9.5 Hz), 5.07 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.5, 17.0 Hz), 5.83 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=6.0, 9.5, 17.0 Hz). *Anal.* Calcd for $C_{12}H_{19}N_3O_2$: C, 60.73; H, 8.07; N, 17.71. Found: C, 60.51; H, 7.82; N, 17.67.

5-Allyl-6-(β-hydroxyethyl)amino-1,3-dimethyluracil (9d) A mixture of **7** (1.07 g, 5 mmol) and β-hydroxyethylamine (3.5 ml) was refluxed for 1 h. The reaction mixture was worked up in a manner similar to that described above for **9a**, giving **9d** (0.72 g, 60%) as colorless needles, mp 80—82 °C. MS m/z: 239 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 2.56 (1H, br, $-CH_2CH_2O\underline{H}$), 3.18 (2H, dt, $-NHC\underline{H}_2CH_2OH$, J=4.5, 5.5 Hz), 3.24 (2H, dt, $-C\underline{H}_2CH=CH_2$, J=1.5, 6.0 Hz), 3.33, 3.43 (3H each, s, NCH_3), 3.75 (2H, br t, $-CH_2C\underline{H}_2OH$, J=4.5 Hz), 4.42 (1H, br t, $-N\underline{H}CH_2CH_2OH$, J=5.5 Hz), 5.03 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.8, 10.0 Hz), 5.09 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 1.8, 17.0 Hz), 5.83 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=6.0, 10.0, 17.0 Hz). *Anal.* Calcd for $C_{11}H_{17}N_3O_3$: C, 55.21; H, 7.16; N, 17.56. Found: C, 55.35; H, 7.06; N, 17.27.

5-Allyl-6-benzylamino-1,3-dimethyluracil (9e) A mixture of **7** (2.14 g, 10 mmol) and benzylamine (4 ml) was refluxed for 2 h, then cooled. Water (15 ml) was added, and the precipitate filtered off and recrystallized from EtOH to give **9e** (2.0 g, 70%) as colorless plates, mp 145—147 °C. MS m/z: 285 (M^+). ^{1}H-NMR (DMSO-d_6) δ: 3.01 (2H, dt, $-C\underline{H}_2CH=CH_2$, J=1.5, 5.8 Hz), 3.13, 3.37 (3H each, s, NCH_3), 4.32 (2H, d, $-C\underline{H}_2C_6H_5$, J=7.0 Hz), 4.91 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 2.0, 10.0 Hz), 4.94 (1H, ddd, $-CH_2CH=C\underline{H}_2$, J=1.5, 2.0, 17.0 Hz), 5.76 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=5.8, 10.0, 17.0 Hz), 5.98 (1H, br t, $-N\underline{H}CH_2C_6H_5$, J=7.0 Hz), 7.23—7.38 (5H, m, $CH_2C_6\underline{H}_5$). *Anal.* Calcd for $C_{16}H_{19}N_3O_2$: C, 67.34; H, 6.71; N, 14.72. Found: C, 67.52; H, 6.82; N, 14.66.

5-Allyl-6-amino-1,3-dimethyluracil (9f) A mixture of **7** (1.07 g, 5 mmol) and 30% ethanolic ammonia (30 ml) was placed in a steel autoclave and heated at 150—160 °C for 7h. After cooling of the mixture, the solvent was removed and water (30 ml) was added to the residue. Crystals were collected by filtration and recrystallized from EtOH to give **9f** (0.8 g, 82%) as colorless needles, mp 108—110 °C. High MS Calcd for $C_9H_{13}N_3O_2$: 195.101. Found: 195.101. ^{1}H-NMR (DMSO-d_6) δ: 3.03 (2H, br d, $-C\underline{H}_2CH=CH_2$, J=6.0 Hz), 3.11, 3.30 (3H each, s, NCH_3), 4.87 (1H, d, $-CH_2CH=C\underline{H}_2$, J=10.0 Hz), 5.01 (1H, d, $-CH_2CH=C\underline{H}_2$, J=17.0 Hz), 5.71 (1H, ddt, $-CH_2C\underline{H}=CH_2$, J=6.0, 10.0, 17.0 Hz), 6.38 (2H, br s, $-NH_2$).

General Procedure for the Catalytic Cyclization of 6-Alkylamino-5-allyl-1,3-dimethyluracils (9a—f) A mixture of $PdCl_2$ $(CH_3CN)_2$ (0.1 eq), benzoquinone (1 eq), LiCl (10 eq), and THF (20 ml/mmol substrate) was stirred for 5 min. The substrate in THF (5 ml/mmol of substrate, 1 eq) was added, and the solution was stirred from 2 to 40 h at room temperature. The THF was removed on a rotary evaporator, and the residue was diluted with water (10 ml/mmol) of substrate). The solution was extracted with $CHCl_3$ (20 ml × 3). The $CHCl_3$ layer was washed three times with 20 ml portions of 1 N aqueous NaOH and dried over Na_2SO_4. The solvent was removed on a rotary evaporator. The residue was purified by preparative TLC (benzene–acetone, 1 : 1) and recrystallized from AcOEt or EtOH.

1,3,6,7-Tetramethylpyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10a) According to the general procedure, the crude product was obtained from **9a** (210 mg, 1 mmol), $PdCl_2$ $(CH_3CN)_2$ (26 mg, 0.1 mmol), benzoquinone (110 mg, 1 mmol), LiCl (420 mg, 10 mmol), and THF (25 ml). The mixture was stirred for 2 h at room temperature. The usual isolation, followed by recrystallization from EtOH gave **10a** (70 mg, 33%) as colorless needles, mp 176—178 °C. High MS Calcd for $C_{10}H_{13}N_3O_2$: 207.101. Found: 207.101. UV λ_{max}^{EtOH} nm (log ε): 218 (4.44), 248 (3.97), 280 (3.90). ^{1}H-NMR ($CDCl_3$) δ: 2.21 (3H, s, CCH_3), 3.36, 3.71, 3.75 (3H each, s, NCH_3), 6.21 (1H, s, 5-H). *Anal.* Calcd for $C_{10}H_{13}N_3O_2 \cdot H_2O$: C, 53.32; H, 6.71; N, 18.65. Found: C, 53.22, H, 6.57; N, 18.68.

7-Ethyl-1,3,6-trimethylpyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10b) According to the general procedure, the crude product was obtained from **9b** (670 mg, 3 mmol), $PdCl_2$ $(CH_3CN)_2$ (78 mg, 0.3 mmol), benzoquinone (330 mg, 3 mmol), LiCl (1.26 g, 30 mmol), and THF (45 ml). The mixture was stirred for 20 h. The usual isolation, followed by preparative TLC (benzene–acetone, 1 : 1) gave **10b** (200 mg, 30%) as colorless needles, mp 154—156 °C. High MS Calcd for $C_{11}H_{15}N_3O_2$: 221.116. Found: 221.117. UV λ_{max}^{EtOH} nm (log ε): 218 (4.34), 248 (3.88), 282 (3.82). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, $CH_2C\underline{H}_3$, J=7.0 Hz), 2.25 (3H, d, CCH_3, J=1.0 Hz), 3.39, 3.76 (3H, each, s, NCH_3), 4.13 (2H, q, $C\underline{H}_2CH_3$, J=7.0 Hz), 6.30 (1H, q, 5-H, J=1.0 Hz). *Anal.* Calcd for $C_{11}H_{15}N_3O_2 \cdot 3/5H_2O$: C, 56.93; H, 7.03; N, 18.10. Found: C, 56.83; H, 6.80; N, 18.03.

1,3,6-Trimethyl-7-propylpyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10c) According to the general procedure, the crude product was obtained from **9c** (711 mg, 3 mmol), $PdCl_2$ $(CH_3CN)_2$ (78 mg, 0.3 mmol), benzoquinone (330 mg, 3 mmol), LiCl (1.26 g, 30 mmol), and THF (45 ml). The mixture was stirred for 30 h. The usual isolation, followed by preparative TLC (benzene–acetone, 1 : 1) gave **10c** (220 mg, 31%) as colorless needles. mp 94—96 °C. High MS Calcd for $C_{12}H_{17}N_3O_2$: 235.131. Found: 235.132. UV λ_{max}^{EtOH} nm (log ε): 218 (4.28), 248 (3.83), 280 (3.79). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, $-CH_2CH_2C\underline{H}_3$, J=7.5 Hz), 1.72 (2H, m, $-CH_2C\underline{H}_2CH_3$), 2.25 (3H, d, CCH_3, J=1.0 Hz), 3.40, 3.74 (3H each, s, NCH_3), 4.00 (2H, t, $-C\underline{H}_2CH_2CH_3$, J=7.5 Hz), 6.30 (1H, q, 5-H, J=1.0 Hz). *Anal.* Calcd for $C_{12}H_{17}N_3O_2 \cdot H_2O$: C, 56.90; H, 7.56; N, 16.58. Found: C, 56.82; H, 7.32; N, 16.60.

7-(β-Hydroxy)ethyl-1,3,6-trimethylpyrrolo[2,3-*d*]pyrimidine-2,4-(1*H*,3*H*)-dione (10d) According to the general procedure, the crude product was obtained from **9d** (239 mg, 1 mmol), $PdCl_2$ $(CH_3CN)_2$ (26 mg, 0.1 mmol), benzoquinone (110 mg, 1 mmol), LiCl (420 mg, 10 mmol), and THF (25 ml). The mixture was stirred for 40 h. The usual isolation (but without washing the $CHCl_3$ layer with 1 N NaOH), followed by preparative TLC (benzene–acetone, 1 : 1) gave **10d** (40 mg, 17%) as colorless needles,

mp 173—175 °C. MS *m/z*: 237 (M^+). UV λ_{max}^{EtOH} nm (log ε): 218 (4.29), 248 (3.86), 280 (3.79). ^{1}H-NMR (DMSO-d_6) δ: 2.23 (3H, d, CCH_3, J=1.0 Hz), 3.20, 3.72 (3H each, s, NCH_3), 3.62 (2H, q, $-NCH_2CH_2OH$, J=5.5 Hz), 4.23 (2H, t, $-NCH_2CH_2OH$, J=5.5 Hz), 5.09 (1H, br t, $-CH_2OH$, J=5.5 Hz), 6.16 (1H, q, 5-H, J=1.0 Hz). *Anal.* Calcd for $C_{11}H_{15}N_3O_3$: C, 55.68; H, 6.35; N, 17.21. Found: C, 55.42; H, 6.37; N, 17.40.

7-Benzyl-1,3,6-trimethylpyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10e) According to the general procedure, the crude product was obtained from **9e** (285 mg, 1 mmol), $PdCl_2$ $(CH_3CN)_2$ (26 mg, 0.1 mmol), benzoquinone (110 mg, 1 mmol), LiCl (420 mg, 10 mmol), and THF (25 ml). The mixture was stirred for 3 h. The usual isolation followed by recrystallization from AcOEt gave **10e** (110 mg, 39%) as colorless plates, mp 160—162 °C. MS *m/z*: 283 (M^+). UV λ_{max}^{EtOH} nm (log ε): 216 (4.62), 246 (4.10), 280 (4.03). ^{1}H-NMR ($CDCl_3$) δ: 2.16 (3H, d, CCH_3, J=1.0 Hz), 3.36, 3.56 (3H each, s, NCH_3), 5.33 (2H, s, $CH_2C_6H_5$), 6.37 (1H, q, 5-H, J=1.0 Hz), 6.85—6.89, 7.23—7.36 (5H, m, $CH_2C_6H_5$). *Anal.* Calcd for $C_{16}H_{17}N_3O_2$: C, 67.82; H, 6.05; N, 14.83. Found: C, 67.61; H, 6.04; N, 14.66.

1,3,6-Trimethylpyrrolo[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (10f) A mixture of **9f** (195 mg, 1 mmol), $PdCl_2$ $(CH_3CN)_2$ (26 mg, 0.1 mmol), benzoquinone (110 mg, 1 mmol), LiCl (420 mg, 10 mmol), and THF (25 ml) was stirred at 40—50 °C for 2 h, then cooled. The precipitate was collected by filtration and purified by column chromatogaphy on silica gel eluted with $CHCl_3$–EtOH (10:1). The fraction containing the product was collected, and concentrated to dryness, then the residue was recrystallized from MeOH to give **10f** (107 mg, 55%) as colorless prisms, mp 268—270 °C. High MS Calcd for $C_9H_{11}N_3O_2$: 193.085. Found: 193.084. UV λ_{max}^{EtOH} nm (log ε): 210 (4.38), 244 (3.84), 278 (3.78). ^{1}H-NMR (DMSO-d_6) δ: 2.21 (3H, d, CCH_3, J=1.0 Hz), 3.19, 3.41 (3H each, s, NCH_3), 6.01 (1H, q, 5-H, J=1.0 Hz), 11.52 (1H, br, NH). *Anal.* Calcd for $C_9H_{11}N_3O_2 \cdot H_2O$: C, 51.17; H, 6.20; N, 19.89. Found: C, 50.95; H, 6.32; N, 19.74.

4-Benzylamino-2,5-dimethyl-2,3-dihydrofuro[3,2-*e*]pyrimidin-6-one (11a) A mixture of **8** (1.0 g, 5 mmol) and benzylamine (1.6 g, 15 mmol) was refluxed for 10 min, then cooled. Water (10 ml) was added, and the precipitate was collected by filtration and recrystallized from EtOH to give **11a** (0.85 g, 63%) as colorless plates, mp 140—142 °C. MS *m/z*: 271 (M^+). UV λ_{max}^{EtOH} nm (log ε): 288 (3.91). ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, d, CCH_3, J=6.0 Hz), 2.55 (1H, dd, 3-H, J=6.0, 13.5 Hz), 3.09 (1H, dd, 3-H, J=8.5, 13.5 Hz), 3.40 (3H, s, NCH_3), 4.57 (2H, br d, $CH_2C_6H_5$, J=6.5 Hz), 4.60—4.72 (1H, m, 2-H), 7.23—7.41 (5H, m, $CH_2C_6H_5$), 7.55 (1H, br t, $NHCH_2$–, J=6.5 Hz). *Anal.* Calcd for $C_{15}H_{17}N_3O_2$: C, 66.40; H, 6.32; N, 15.49. Found: C, 66.20; H, 6.29; N, 15.42.

X-Ray Crystallographic Analysis of 11a A crystal of **11a** with the dimensions of 0.7×0.3×0.3 mm^3 was used for the analysis. The cell dimensions and diffraction intensities were measured with a Rigaku four-circle diffractometer (AFC-5R), using graphitemonochromated Mo Kα radiation (λ=0.71069 Å) at 20±1 °C.

Crystal Data: $C_{15}H_{19}N_3O_3$, M_r 289.33, monoclinic, space group $P2_1/a$, a=10.48 (6), b=8.61 (1), c=16.29 (7) Å, β=92.4 (4)°, V=0000 (1) Å^3, Z=4, D_c=1.308 g/cm^3, μ (Mo Kα)=0.87 cm^{-1}. The ω–2θ scan mode with a scan rate of 16°/min was employed with the ω scan range (1.20+0.30 tanθ)°. A total of 3686 reflections were collected up to 2θ of 55.1°. The collected reflection intensities were corrected for Lorentz and polarization factors, but not for absorption. The structures were solved by direct methods using the program MITHRIL.[13] The non-hydrogen atoms were refined by the full-matrix least-squares method with anisotropic temperature factors. In the difference Fourier map, one molecule of water was found. The positions of all hydrogen atoms were calculated but not refined. At the final stage of refinement, 2018 reflections with $|F_o| > 3\sigma(|F_o|)$ out of 3686 unique reflections were used. Final R was 0.045 (R_w=0.052). The function minimized was $\sum_w(|F_o|-|F_c|)^2$, where w was taken as $4F_o^2/\sigma^2(F_o^2)$. Atomic scattering factors were taken from the International Tables for X-ray Crystallography (1974).[14] No peak larger than 0.23 eÅ^{-3} was found in the last difference electron density map. All calculations were performed using the TEXSAN[15] crystallographic software package of Molecular Structure Corporation.

The perspective drawing of **11a** and the numbering scheme of atoms are shown in Fig. 2. The final positional and thermal parameters are listed in Table I. The bond distances and angles are presented in Table II and III, respectively.

The bond lengths and angles are within normal ranges. As shown in Fig. 2, the methyl group attached to the 2-position and the phenyl group are situated on the same side of the plane of the furopyrimidine skeleton.

2,5-Dimethyl-4-methylamino-2,3-dihydrofuro[3,2-*e*]pyrimidin-6-one (11b) A mixture of **8** (0.60 g, 3 mmol) and a 40% aqueous solution of methylamine (6 ml) was stirred under reflux for 1 h. After evaporation of the solvent, the residue was subjected to silica gel column chromatography with $CHCl_3$–EtOH (5:1) and the product was recrystallized from $CHCl_3$–AcOEt (2:1) to give **11b** (0.38 g, 65%) as a white powder, mp 172—173 °C. MS *m/z*: 195 (M^+). UV λ_{max}^{EtOH} nm (log ε): 286 (4.13). ^{1}H-NMR (DMSO-d_6) δ: 1.33 (3H, d, CCH_3, J=6.0 Hz), 2.92 (1H, dd, 3-H, J=6.5,

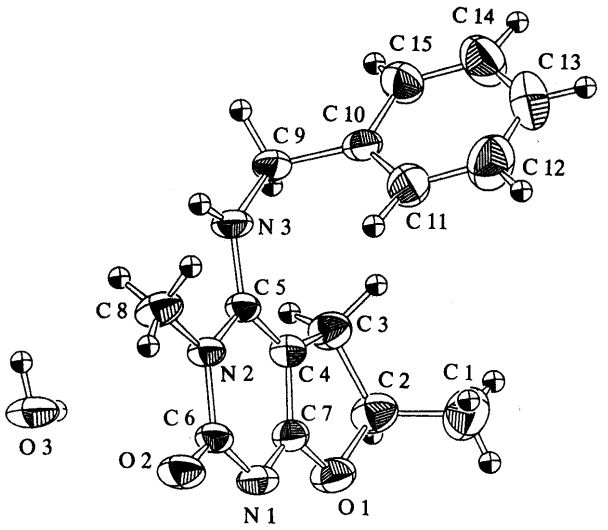


Fig. 2. Perspective Drawing of the Molecule **11a** with the Atomic Numbering

TABLE I. The Positional Parameters and Equivalent Isotropic Thermal Parameters with Their Estimated Standard Deviations in Parentheses

Atom	x (×10^4)	y (×10^4)	z (×10^4)	B_{eq} (Å^2)
O1	5113 (2)	1907 (2)	7370 (1)	3.71 (8)
O2	1582 (2)	1454 (2)	5603 (1)	3.76 (8)
O3	4121 (2)	3356 (2)	4426 (1)	4.3 (1)
N1	3353 (2)	1553 (2)	6483 (1)	3.10 (8)
N2	2255 (2)	3846 (2)	6025 (1)	2.52 (8)
N3	2801 (2)	6339 (2)	6446 (1)	3.08 (9)
C1	5275 (5)	2913 (5)	8739 (2)	6.5 (2)
C2	5686 (3)	3148 (3)	7884 (2)	3.9 (1)
C3	5189 (3)	4682 (3)	7494 (2)	3.5 (1)
C4	4049 (2)	4117 (2)	6970 (1)	2.7 (1)
C5	3066 (2)	4811 (2)	6496 (1)	2.45 (9)
C6	2387 (2)	2220 (2)	6024 (1)	2.8 (1)
C7	4111 (2)	2513 (2)	6913 (1)	2.7 (1)
C8	1189 (3)	4505 (3)	5520 (2)	3.6 (1)
C9	3465 (3)	7531 (3)	6943 (2)	3.0 (1)
C10	3015 (2)	7684 (2)	7806 (1)	2.9 (1)
C11	2142 (3)	6674 (3)	8148 (2)	4.2 (1)
C12	1766 (3)	6899 (4)	8937 (2)	5.7 (2)
C13	2261 (4)	8122 (4)	9409 (2)	5.8 (2)
C14	3109 (3)	9130 (4)	9066 (2)	5.5 (2)
C15	3479 (3)	8911 (3)	8290 (2)	4.2 (1)

TABLE II. Bond Lengths (Å) with Their Standard Deviations in Parentheses

O1–C2	1.470 (6)	C2–C3	1.548 (6)
O1–C7	1.366 (7)	C3–C4	1.520 (8)
O2–C6	1.253 (6)	C4–C5	1.397 (7)
N1–C6	1.361 (7)	C4–C7	1.386 (4)
N1–C7	1.326 (6)	C9–C10	1.507 (7)
N2–C5	1.395 (7)	C10–C11	1.396 (7)
N2–C6	1.407 (3)	C10–C15	1.393 (6)
N2–C8	1.474 (8)	C11–C12	1.374 (7)
N3–C5	1.347 (4)	C12–C13	1.391 (7)
N3–C9	1.465 (6)	C13–C14	1.378 (7)
C1–C2	1.490 (8)	C14–C15	1.351 (7)

TABLE III. Bond Angles (°) with Their Standard Deviations in Parentheses

C2–O1–C7	108.6 (3)	O2–C6–N1	123.2 (3)
C6–N1–C7	116.3 (3)	O2–C6–N2	117.4 (3)
C5–N2–C6	122.4 (3)	N1–C6–N2	119.4 (3)
C5–N2–C8	120.5 (3)	O1–C7–N1	118.6 (3)
C6–N2–C8	117.1 (3)	O1–C7–C4	112.5 (3)
C5–N3–C9	124.0 (3)	N1–C7–C4	128.9 (3)
O1–C2–C1	107.8 (4)	N3–C9–C10	114.5 (4)
O1–C2–C3	105.3 (3)	C9–C10–C11	123.6 (4)
C1–C2–C3	113.3 (4)	C9–C10–C15	118.5 (4)
C2–C3–C4	101.6 (4)	C11–C10–C15	117.9 (4)
C3–C4–C5	136.0 (3)	C10–C11–C12	120.3 (4)
C3–C4–C7	108.6 (4)	C11–C12–C13	120.5 (4)
C5–C4–C7	115.2 (3)	C12–C13–C14	119.1 (4)
N2–C5–N3	115.5 (3)	C13–C14–C15	120.5 (4)
N2–C5–C4	117.8 (3)	C10–C15–C14	121.8 (4)
N3–C5–C4	126.7 (3)		

13.5 Hz), 2.98 (3H, d, NHC$\underline{H}_3$, J=4.0 Hz), 3.24 (3H, s, NCH_3), 3.50 (1H, dd, 3-H, J=8.5, 13.5 Hz), 4.72—4.84 (1H, m, 2-H), 7.10 (1H, br q, N$\underline{H}CH_3$, J=4.0 Hz). *Anal.* Calcd for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.52. Found: C, 55.19; H, 6.71; N, 21.27.

4-Amino-2,5-dimethyl-2,3-dihydrofuro[3,2-*e*]pyrimidin-6-one (11c) A mixture of **8** (0.40 g, 2 mmol) and a 25% aqueous solution of ammonia (10 ml) was stirred under reflux for 3 h. After concentration of the mixture, the residue was purified by silica gel column chromatography, eluted with $CHCl_3$–EtOH (5:1), and the product was recrystallized from $CHCl_3$–EtOH (10:1) to give **11c** (0.22 g, 61%) as a white powder, mp 122—124 °C. High MS Calcd for $C_8H_{11}N_3O_2$: 181.085. Found: 181.085. UV λ_{max}^{EtOH} nm (log ε): 280 (4.12). ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, d, CCH_3, J=6.0 Hz), 2.40 (1H, dd, 3-H, J=6.0, 14.0 Hz), 3.01 (1H, dd, 3-H, J=8.5, 14.0 Hz), 3.22 (3H, s, NCH_3), 4.79—4.91 (1H, m, 2-H), 7.10 (2H, s, NH_2). *Anal.* Calcd for $C_8H_{11}N_3O_2 \cdot 2H_2O$: C, 44.23; H, 6.95; N, 19.34. Found: C, 44.27; H, 6.85; N, 19.21.

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Study on the Bile Salt, Sodium Scymnol Sulfate, from *Lamna ditropis*. III. The Structures of a New Sodium Scymnol Sulfate and New Anhydroscymnols[1)]

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Two sodium scymnol sulfates, 1 and 2, were isolated by two steps of chromatography from the bile of *Lamna ditropis*. Compound 2 was identified as (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate and the structure of 1 was determined to be (24*R*,25*R*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate, based on the spectral data and chemical transformations. Based on the physical data, the structures of two new anhydroscymnols, 3 and 4, prepared from 1 by alkaline degradation with aqueous potassium hydroxide, were established as (24*R*,25*R*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol and (24*R*)-(+)-26,27-epoxy-5β-cholestane-3α,7α,12α,24-tetrol, respectively. The other shark bile constituents were also analyzed, and the hydrolysis of the sulfate ester function of 1 and 2 was examined.

Keywords sodium scymnol sulfate; *Lamna ditropis*; *Rhizoprionodon acutus*; *Chlamydoselachus anguineus*; anhydroscymnol

Sodium scymnol sulfate has been isolated from the biles of sharks, *Scymnus borealis*,[2)] *Galeocerdo articus*,[3)] *Squalus acanthias*[4)] and *Rhizoprionodon acutus*,[5)] and also rays, *Dasyatis akajei*[6)] and *Raia batis*,[4)] and is regarded as a typical component of the bile of all *Elasmobranch* fishes.

In the preceding paper,[1)] we reported the structure determination of sodium scymnol sulfate (**2**) isolated from the bile of *Rhizoprionodon acutus*,[2,5)] and scymnol (**5**)[5,6)] and anhydroscymnol (**6**),[2-5,7,8)] prepared from **2**, as (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate, (24*R*)-(+)-5β-cholestane-3α,7α,12α,24,26,27-hexol and (24*R*,25*S*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol, respectively, on the basis of X-ray diffraction analyses, physicochemical data and chemical transformations.

In the course of our study on sodium scymnol sulfate, we found that there are two sodium scymnol sulfates in the bile of *Lamna ditropis*. This paper deals with the isolation of their salts from the bile of *Lamna ditropis* and presents details of their structural determinations. The analyses of the shark biles and the transformation of sodium scymnol sulfate to scymnol are also described.

The shark, *Lamna ditropis*, inhabits the cold seas near Tohoku and Hokkaido in Japan and Alaska and California in the United States of America. To our knowledge, there has been no previous study on its bile constituents.

Isolation of sodium scymnol sulfates from the bile of *Lamna ditropis* was achieved by two steps of column chromatography (HP-20 and high-performance liquid chromatography (HPLC)). The procedures are summarized in Chart 1. The bile was applied to an HP-20 column after being defatted with ethyl acetate. Much inorganic material and impurities were removed on this column. There are two main constituents, tentatively named compounds I and II, in the methanolic eluate. As shown in Fig. 1, both compounds were purified by HPLC, though with difficulty: 64.3 mg of compound I was isolated from the first peak as an amorphous powder, while 39.7 mg of compound II was isolated from the second peak, also as an amorphous powder.

These compounds have not yet been crystallized. The spectral data indicated compound II to be (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**2**).[1)] The identification was confirmed by chemical

lyophilized bile of *Lamna ditropis* (2.01 g)
- 1) dissolved in H_2O (200 ml)
- 2) washed with AcOEt (200 ml × 2)

H_2O layer
- 1) diluted with H_2O (1200 ml)
- 2) HP-20 c.c. eluted with 1) H_2O (1500 ml) and 2) MeOH (400 ml)

MeOH eluate (352 mg)
- HPLC: YMC-pack A-324 (ODS)
- mobile phase: 31.5% CH_3CN–0.1 N sodium phosphate buffer (pH 6.70)

1 (64.3 mg) **2** (39.7 mg)

( ) indicates yield. c.c., column chromatography.

Chart 1. Isolation Procedure for Compounds I (**1**) and II (**2**)

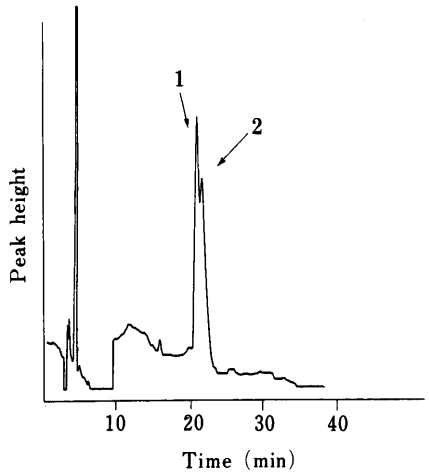


Fig. 1. HPLC Chromatogram of the MeOH Eluate Obtained from the Bile of *Lamna ditropis*

HPLC condition: column, YMC-pack A-324 (10 × 300 mm); mobile phase, 31.5% AcCN–0.1 N sodium phosphate buffer (pH 6.7); flow rate, 3.0 ml/min; detection, RI.

transformation and direct comparison of the spectral data (infrared (IR), nuclear magnetic resonance (NMR) and secondary ionization mass spectrum (SIMS)) with those of an authentic sample, obtained from the bile of *Rhizoprionodon acutus*.[1,5]

Compound I afforded scymnol (**5**) on nonaqueous hydrolysis of its O-acetylated derivative with trichloroacetic acid, followed by alkaline hydrolysis with aqueous potassium hydroxide. From direct elementary analysis and SIMS of compound I (**1**), its molecular formula was determined to be $C_{27}H_{47}NaO_9S$. In its IR spectrum there was an absorption band, assigned to the sulfate ester function, at 1230 cm^{-1}. From a detailed comparison of the physical data of **1** with those of **2**, it was indicated that **1** is sodium scymnol sulfate. The ^1H- and ^{13}C-NMR signals of the side chains of **1** and **2** are shown in Table I. There is no significant difference in the chemical shifts of the corresponding carbons between **1** and **2**. Further, the chemical shift of C-27, shown in Table I, indicates that the hydroxyl group at C-27 is esterified with SO_3Na in **1**. Among the proton signals, four signals at δ 4.18 (1H, dd), 4.14 (1H, dd), 3.82 (1H, dd) and 3.71 (1H, dd) were assignable to 27-H_A, 27-H_B, 26-H_A and 26-H_B. It can be seen that $J_{25,26}$ of **1** is similar to $J_{25,27}$ of **2**, and $J_{25,27}$ of **1** is similar to $J_{25,26}$ of **2**. These results suggest that this compound is the 25-epimer of **2**, namely, (24*R*,25*R*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**1**). This was confirmed in the following way.

TABLE I. 500 MHz ^1H- and ^{13}C-NMR Data for the Side Chains of **1** and **2** in D_2O (Coupling Constant in Parenthesis)

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
20	1.40 m	38.6 d	1.41 m	38.6 t
21	1.02 d (6.1)	19.9 q	1.02 d (6.3)	19.9 q
22	1.45 m	34.5 t	1.46 m	34.6 t
23	1.46 m	32.9 t	1.48 m	33.1 t
	1.68 m		1.70 m	
24	3.77 m	73.1 d	3.75 m	72.9 d
25	1.92 m	48.3 d	1.95 m	48.5 d
26	3.71 dd	61.7 t	3.69 dd	62.3 t
	(6.7, 11.5 (*gem*))		(6.4, 11.4 (*gem*))	
	3.82 dd		3.74 dd	
	(4.7, 11.5 (*gem*))		(5.5, 11.4 (*gem*))	
27	4.14 dd	69.7 t	4.15 dd	68.9 t
	(6.2, 10.1 (*gem*))		(6.5, 10.0 (*gem*))	
	4.18 dd		4.25 dd	
	(5.7, 10.1 (*gem*))		(4.5, 10.0 (*gem*))	

δ values in ppm and coupling constants in Hz. Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as d, t and q.

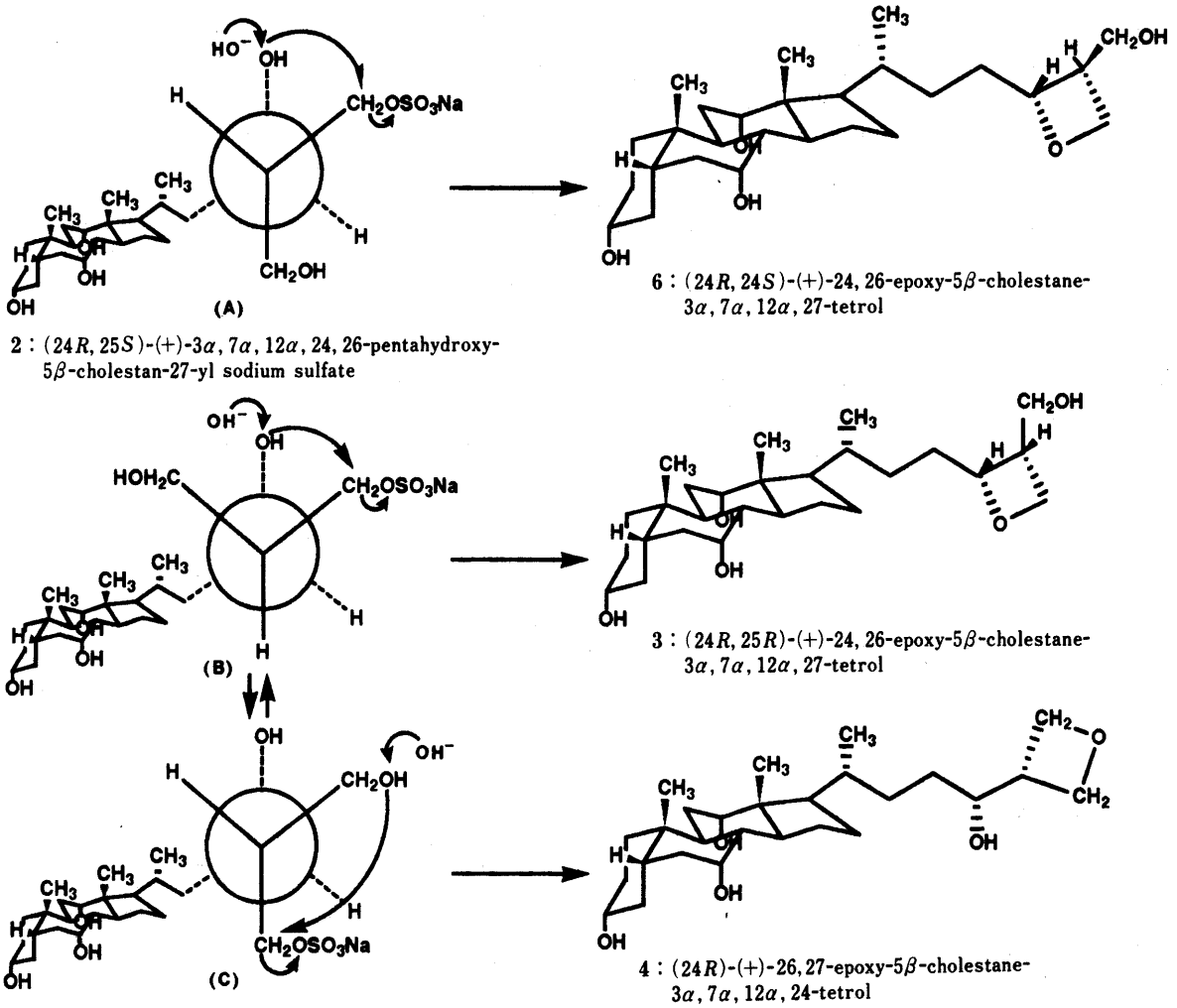


Chart 2. Mechanisms of Formation of Anhydroscymnols (**3**, **4** and **6**) from Sodium Scymnol Sulfates, **1** and **2**

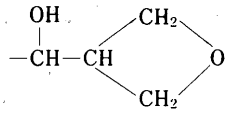
TABLE II. 500 MHz ^{1}H- and ^{13}C-NMR Data for the Side Chains of 3—6 (Coupling Constants in Parenthesis)

Position	3		4		5		6	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
20	1.40 m	36.6 d	1.74 m	36.2 d	1.42 m	37.8 d	1.56 m	36.6 d
21	1.02 d (6.6)	18.1 q	1.24 d (6.2)	17.9 q	1.07 d (6.0)	18.9 q	1.03 (6.2)	18.2 q
22	1.52 m	32.0 t	1.62 m	32.3 t	1.47 m	34.0 t	1.58 m	31.6 t
23	1.51 m	29.4 t	1.47 m	32.0 t	1.44 m	33.0 t	1.60 m	34.6 t
	1.80 m		1.61 m		1.66 m		1.81 m	
24	4.71 ddd (5.1, 7.6, 8.7)	84.6 d	4.04 m	72.6 d	3.65 m	73.0 d	4.41 ddd (6.1, 6.1, 6.1)	85.9 d
25	3.02 ddddd (5.7, 7.4, 7.6, 7.6, 8.0)	40.7 d	3.16 ddddd (6.1, 6.1, 7.9, 7.9, 8.1)	41.9 d	1.91 m	49.0 d	2.76 ddddd (6.0, 6.1, 6.2, 6.2, 8.1)	44.2 d
26	4.11 dd (5.7, 5.7)	71.4 t	4.70 (6.1, 6.1)	74.7 t	3.64 m	62.0 t	4.32 dd (6.2, 6.2)	70.5 t
	4.57 dd (5.7, 8.0)		5.01 (6.1, 6.1)				4.46 dd (6.2, 8.1)	
27	3.79 dd (7.6, 10.9)	61.5 t	4.78 (5.7, 7.9)	73.9 t	3.70 m	62.7 t	3.72 dd (6.2, 6.2)	64.1 t
	3.92 dd (7.4, 10.9)		4.88 (5.7, 7.9)				3.76 dd (6.0, 6.2)	

δ values in ppm and coupling constants in Hz. Multiplicities of carbon signals were determined by means of the DEPT method and are indicated as d, t and q. Solvents: acetone-d_6 for **3** and **6**, pyridine-d_5 for **4** and MeOH for **5**.

Alkaline degradation of **1** with aqueous potassium hydroxide gave two anhydroscymnols, **3** and **4**, in 38 and 30% yields, respectively, as shown in Chart 2. The chemical shifts of the C-20 to C-27 carbons in the side chain and the proton spin–spin coupling constants for **3**, **4**, **5** and **6**[1] were obtained from the ^{1}H- and ^{13}C-NMR, and ^{1}H–^{1}H and ^{13}C–^{1}H correlation spectroscopy (COSY) NMR spectra, and are summarized in Table II. A comparison of the chemical shifts of the C-24 to C-27 carbons of **3** with those of **6** indicates that compound **3** has a 24,26-epoxy ring in the side chain (Table II). The coupling constant $J_{24,25}$ of **3**, 7.6 Hz, indicates that the relative configuration of the protons is *cis*.[9] From the above analyses and physicochemical data of **3**, its structure is determined to be (24*R*,25*R*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol.

As can be seen in Table II, in the ^{13}C-NMR spectrum of **4** the signals of C-24, C-26 and C-27 appear at 72.6, 74.7 and 73.9 ppm, and in that of **5**, at 73.0, 62.7 and 62.0 ppm. This indicates that compound **4** has the following partial structure. Thus, compound **4** is determined to be (24*R*)-



(+)-26,27-epoxy-5β-cholestane-3α,7α,12α,24-tetrol.

Alkaline degradation of **2** with aqueous potassium hydroxide gave **6** in 76% yield, while that of **1** afforded **3** and **4** in 38 and 30% yields, respectively. The formation of the trimethylene oxide ring of **3**, **4** and **6** can be accounted for by elimination of the OSO_3Na group due to the nucleophilic attack of hydroxyl oxygen at C-24 or C-26 of **2** or **1** during alkaline degradation. As a result, compound **1** is concluded to be (24*R*,25*R*)-(+)-3α,7α,12α;24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**1**).

The formation of anhydroscymnol **6** from **2** and that of **3** and **4** from **1** could be explained in the following way. In the case of **2**, the alkaline degradation reaction proceeds *via* the stable transition state A to afford only **6** by the attack of hydroxyl oxygen at C-24 on the C-27 carbon (see Chart 2). On the other hand, since the stabilities of the two states B and C for **1** are almost equal, it gave nearly equal amounts of two anhydroscymnols, **3** and **4**.

It is very interesting in connection with the biological evolution of the shark that there are two main constituents, sodium scymnol sulfates, **1** and **2**, in the bile of *Lamna ditropis*, but only one sodium scymnol sulfate (**2**) in the bile of *Rhizoprionodon acutus*. So, we carefully analyzed the biles of several sharks, *Rhizoprionodon acutus*, *Chlamydoselachus anguineus* GARMAN and *Glyphis glaucus*. Sodium scymnol sulfate (**1**) was found only in the bile of *Lamna ditropis*. This is noteworthy, because *Chlamydoselachus anguineus* GARMAN has a primitive character from an evolutional viewpoint.

As noted, scymnol (**5**) was obtained from sodium scymnol sulfates, **1** and **2**, in moderate yield (60—64%), in the manner reported previously.[5,6] Upon investigation of the hydrolysis of the sulfate ester function of sodium scymnol sulfate, it was found that treatment of sodium scymnol sulfate with Dowex 50W-X8 in methanol at room temperature afforded **5** quantitatively, and also heating of a solution of sodium scymnol sulfate in pyridine–dioxane (1 : 1) gave **5** in very high yield.

In summary, we have isolated two sodium scymnol sulfates from the bile of *Lamna ditropis*, and confirmed their structures as (24*R*,25*R*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**1**), which exists only in the gallbladder of *Lamna ditropis* to our knowledge, and (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate (**2**). Compound **1** afforded two anhydroscymnols, (24*R*,25*R*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol (**3**) and (24*R*)-(+)-26,27-epoxy-5β-cholestane-3α,7α,12α,24-tetrol (**4**), on alkaline degradation with aqueous potassium hydroxide.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. IR spectra were taken on a JASCO IRA-2 grating IR spectrometer. Optical rotation was measured with a JASCO DIP-140. Chemical ionization mass spectra (CI-MS) and high-resolution mass spectra (MS) were recorded on a Hitachi M-80A instrument and SIMS were obtained on a Hitachi M-80B machine, using diethanolamine as the matrix. NMR spectra were recorded on JEOL GX-500 and Brucker AM-500 spectrometers using tetramethylsilane (TMS) as an internal standard. Chemical shifts are recorded in δ values (ppm) and coupling constants in herts (Hz). Multiplicities of ^{13}C-NMR signals were determined by means of the distortionless enhancement by

polarization transfer (DEPT) method. ^1H–^1H COSY and ^1H–^{13}C COSY spectra were obtained with the JEOL and Brucker standard pulse sequences and data processings were performed with the standard software.

Isolation of Sodium Scymnol Sulfates Material: Gallbladders, obtained from sharks, *Lamna ditropis* (*ca.* 20 kg), collected in July 1989 off the coast of Sanriku, Japan, were homogenized and the homogenate was freeze-dried. This material (2.01 g) was used as a source.

Isolation of Sodium Scymnol Sulfates **1** and **2** from the Bile of *Lamna ditropis*: The lyophilized bile (2.01 g) of *Lamna ditropis* was dissolved in H_2O (20 ml), and defatted with AcOEt (20 ml × 2). The H_2O layer was diluted with H_2O (120 ml) and applied to an HP-20 (25 ml) column. The column was eluted with H_2O (160 ml) and then with MeOH (40 ml). Purification of the MeOH eluate (352 mg) by HPLC yielded 64.3 mg of sodium scymnol sulfate (**1**) and 39.7 mg of sodium scymnol sulfate (**2**). The conditions for HPLC were as follows: column, YMC-pack A-324 (ODS) 10 × 300 mm; flow rate, 3 ml/min; mobile phase, 31.5% CH_3CN–0.1 N sodium phosphate buffer (pH 6.70); detector, refraction index (RI). Compounds **1** and **2** gave the following physical data.

Compound **1**: White amorphous powder, $[\alpha]_D^{25}$ 33.8° (c=0.5, MeOH). *Anal.* Calcd for $C_{27}H_{47}NaO_9S$: C, 56.82; H, 8.30; Na, 4.03; S, 5.62. Found: C, 56.71; H, 8.43; Na, 3.79; S, 5.55. SIMS mass m/z: 759 $[C_{27}H_{47}O_9S \cdot 2HN(C_2H_6O)_2]$, 654 $[C_{27}H_{47}O_9S \cdot HN(C_2H_6O)_2]$, 574 $[C_{27}H_{47}O_6 \cdot HN(C_2H_6O)_2]$. IR ν_{max}^{KBr} cm^{-1}: 3420, 2950, 1470, 1380, 1230, 1070, 980, 910, 810. ^1H-NMR (in D_2O) δ: 4.18 (1H, dd, J=5.7, 10.1 Hz, 27-H_A), 4.14 (1H, dd, J=6.2, 10.1 Hz, 27-H_B), 4.07—4.04 (1H, m, 12-H), 3.91—3.87 (1H, m, 7-H), 3.82 (1H, dd, J=4.7, 11.5 Hz, 26-H_A), 3.77 (1H, m, 24-H), 3.71 (1H, dd, J=6.7, 11.5 Hz, 26-H_B), 3.57—3.45 (1H, m, 3-H), 2.20—1.24 (25H, m), 1.02 (3H, d, J=6.1 Hz, 21-H), 0.92 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in D_2O) δ: 75.8 (C-12, d), 74.3 (C-3, d), 73.1 (C-24, d), 70.9 (C-7, d), 69.7 (C-27, t), 61.7 (C-26, t), 49.4 (C-17, d), 49.0 (C-13, s), 48.3 (C-25, d), 44.3 (C-14, d), 44.1 (C-5, d), 42.4 (C-8, d), 41.2 (C-4, t), 38.6 (C-20, d), 37.9 (C-1, t), 37.3 (C-10, s), 36.9 (C-6, t), 34.5 (C-22, t), 32.9 (C-23, t), 32.0 (C-2, t), 30.7 (C-11, t), 30.3 (C-16, t), 29.2 (C-9, d), 26.0 (C-15, t), 25.2 (C-19, q), 19.9 (C-21, q), 15.2 (C-18, q).

Compound **2**: White amorphous powder, $[\alpha]_D^{25}$ 21.75° (c=0.5, MeOH). *Anal.* Calcd for $C_{27}H_{47}NaO_9S$: C, 56.82; H, 8.30; Na, 4.03; S, 5.62. Found: C, 56.89; H, 8.59; Na, 4.23; S, 5.65. SIMS mass m/z: 654 $[C_{27}H_{47}O_9S \cdot HN(C_2H_6O)_2]$, 574 $[C_{27}H_{47}O_6 \cdot HN(C_2H_6O)_2]$. IR ν_{max}^{KBr} cm^{-1}: 3420, 2950, 1470, 1380, 1230, 1070, 980, 910, 810. ^1H-NMR (in D_2O) δ: 4.25 (1H, dd, J=4.5, 10.0 Hz, 27-H_A), 4.15 (1H, dd, J=6.5, 10.0 Hz, 27-H_B), 4.07—4.04 (1H, m, 12-H), 3.91—3.87 (1H, m, 7-H), 3.77—3.73 (1H, m, 24-H), 3.74 (1H, dd, J=5.5, 11.4 Hz, 26-H_A), 3.69 (1H, dd, J=6.4, 11.4 Hz, 26-H_B), 3.52—3.45 (1H, m, 3-H), 2.21—2.06 (2H, m), 2.07—1.26 (21H, m), 1.12—0.95 (2H, m), 1.02 (3H, d, J=6.3 Hz, 21-H), 0.91 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in D_2O) δ: 75.7 (C-12, d), 74.3 (C-3, d), 72.9 (C-24, d), 70.9 (C-7, d), 68.9 (C-27, t), 62.3 (C-26, t), 49.3 (C-17, d), 49.0 (C-13, s), 48.5 (C-25, d), 44.2 (C-14, d), 44.1 (C-5, d), 42.4 (C-8, d), 41.1 (C-4, t), 38.6 (C-20, d), 37.9 (C-1, t), 37.3 (C-10, s), 36.8 (C-6, t), 34.6 (C-22, t), 33.1 (C-23, t), 32.0 (C-2, t), 30.7 (C-11, t), 30.3 (C-16, t), 29.2 (C-9, d), 26.0 (C-15, t), 25.2 (C-19, q), 19.9 (C-21, q), 15.2 (C-18, q). This compound was identified as (24*R*,25*S*)-(+)-3α,7α,12α,24,26-pentahydroxy-5β-cholestan-27-yl sodium sulfate by direct comparison with authentic sodium scymnol sulfate, which gave the following mass data. SIMS mass m/z: 654 $[C_{27}H_{47}O_9S \cdot HN(C_2H_6O)_2]$, 574 $[C_{27}H_{47}O_6 \cdot HN(C_2H_6O)_2]$.[5]

Acid Hydrolysis of 1 with Trichloroacetic Acid A mixture of 21 mg of **1** and 0.3 ml of acetic acid and acetic anhydride (1 : 1) was refluxed for 3 h. The solvent was removed *in vacuo*, and the residue was dissolved in 0.2 ml of dry dioxane. To this solution, 0.1 ml of freshly distilled trichloroacetic acid was added. The mixture was left for 7 d at room temperature, then diluted with 0.1 ml of aqueous 1 M $BaCl_2$ to yield 8 mg of $BaSO_4$. After removal of the precipitates by filtration, the filtrate was concentrated under reduced pressure. A solution of 50 mg of potassium hydroxide in 0.5 ml of MeOH was added to the concentrate and the mixture was refluxed for 3 h. The excess MeOH was removed and the residue was diluted with 15 ml of H_2O, then neutralized with diluted hydrochloric acid, and extracted twice with 5 ml of AcOEt and *n*-butanol (1 : 1). The organic phase was washed with saturated aqueous NaCl and dried with $MgSO_4$. The concentrate was chromatographed with $CHCl_3$–MeOH–H_2O (60 : 25 : 5) as an eluent to afford 11 mg of **5**. Upon crystallization of this product from AcOEt and MeOH, colorless plates were obtained. The physical properties of **5** are as follows.

Compound **5**: Colorless plates, mp 195—196 °C (lit. 190 °C),[3] $[\alpha]_D^{25}$ 40.4° (c=0.5, MeOH), 34.2° (c=0.9, EtOH). *Anal.* Calcd for $C_{27}H_{48}O_6 \cdot H_2O$: C, 66.63; H, 10.36. Found: C, 66.54; H, 9.90. High-resolution MS: 432.3221 (error −1.2 mMU) for $C_{27}H_{44}O_4$($C_{27}H_{48}O_6$ − $2H_2O$). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1480, 1380, 1080, 1040, 980, 920. ^1H-NMR (in MeOH-d_4) δ: 3.97—3.93 (1H, m, 12-H), 3.82—3.62 (6H, m, 7, 24, 26, 26, 27 and 27-H), 3.37 (1H, dddd, J=6.2, 6.2, 11.0, 11.0 Hz, 3-H), 2.36—2.22 (2H, m, 4 and 9-H), 2.02—1.24 (21H, m), 1.15—1.05 (1H, m, 15-H), 1.07 (3H, d, J=6.1 Hz, 21-H), 1.01—0.95 (1H, m, 1-H), 0.91 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in MeOH-d_4) δ: 74.8 (C-12, d), 73.5 (C-3, d), 73.0 (C-24, d), 69.8 (C-7, d), 62.7 (C-27, t), 62.0 (C-26, t), 50.0 (C-17, d), 49.0 (C-25, d), 48.1 (C-13, s), 43.8 (C-14, d), 43.6 (C-5, d), 41.7 (C-8, d), 41.1 (C-4, t), 37.8 (C-20, d), 37.2 (C-1, t), 36.6 (C-10, s), 36.5 (C-6, t), 34.0 (C-22, t), 33.0 (C-23, t), 31.9 (C-2, t), 30.3 (C-11, t), 29.5 (C-16, t), 28.5 (C-9, d), 25.0 (C-15, t), 24.0 (C-19, q), 18.9 (C-21, q), 13.8 (C-18, q). From these data and by a comparison of them with those of the authentic sample, this compound was identified as (24*R*)-(+)-5β-cholestane-3α,7α,12α,24,26,27-hexol (scymnol).[5]

Acid Hydrolysis of 2 with Trichloroacetic Acid Pure scymnol (11 mg) was obtained from compound **2** (21 mg), according to the same procedures as described above.

Hydrolysis of 1 with Dowex 50W-X8 in MeOH Dowex 50W-X8 (20 mg, 100—200 mesh) was added to a solution of 22.8 mg of sodium scymnol sulfate (**1**) (0.04 mmol) in 0.5 ml of MeOH, and the mixture was stirred for 12 h. After removal of the resin by filtration, the filtrate was concentrated under reduced pressure. The residue was recrystallized from AcOEt and MeOH to afford 18.5 mg of **5** as colorless plates.

Heating of 2 in Pyridine–Dioxane (1 : 1) A solution of sodium scymnol sulfate (22.8 mg) in 0.5 ml of pyridine–dioxane (1 : 1) was refluxed for 12 h, and the reaction mixture was concentrated under reduced pressure. The reaction product was purified by silica gel column chromatography with the lower layer of $CHCl_3$–MeOH–H_2O (60 : 25 : 5) as a solvent, and recrystallized from AcOEt and MeOH to give 18.1 mg of **5** as colorless plates.

Alkaline Degradation of 1 with Potassium Hydroxide In a sealed tube, 15 mg of **1** in 0.5 ml of 2.5 N aqueous potassium hydroxide was heated at 120 °C for 16 h. After saturation of the resultant solution with NaCl, the products were extracted with 1 ml of AcOEt and *n*-butanol (1 : 1) three times. The organic layer was washed with H_2O and saturated NaCl, and the solvent was distilled off under reduced pressure. The concentrate was purified by HPLC to afford 4.2 mg of **3** and 3.5 mg of **4**. The conditions for HPLC were the same as those described above. Recrystallization of **3** from aqueous MeOH gave 3.4 mg of colorless needles (mp 194—195 °C) and that of **4** from AcOEt–MeOH gave 2.2 mg of colorless needles (mp 199—200 °C). Compounds **3** and **4** have the following physical properties.

Compound **3**: Colorless needles, mp 194—195 °C, $[\alpha]_D^{25}$ 27.7° (c=0.7, MeOH). *Anal.* Calcd for $C_{27}H_{46}O_5 \cdot 1/2H_2O$: C, 70.59; H, 10.45. Found: C, 70.65; H, 10.35. High-resolution MS: 432.3258 (error +2.3 mMU) for $C_{27}H_{44}O_4$ ($C_{27}H_{46}O_5 - H_2O$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2875, 1480, 1380, 1080, 1040, 1020, 980, 955, 920, 860. ^1H-NMR (in acetone-d_6) δ: 4.71 (1H, ddd, J=5.1, 7.6, 8.7 Hz, 24-H), 4.57 (1H, dd, J=5.7, 8.0 Hz, 26-H_A), 4.11 (1H, dd, J=5.7, 5.7 Hz, 26-H_B), 3.98—3.95 (1H, m, 12-H), 3.92 (1H, dd, J=7.4, 10.9 Hz, 27-H_A), 3.82—3.79 (1H, m, 7-H), 3.79 (1H, dd, J=7.6, 10.9 Hz, 27-H_B), 3.63 (1H, dd, J=4.9, 4.9 Hz), 3.37—3.29 (2H, m), 3.26 (1H, d, J=4.2 Hz), 3.07 (1H, d, J=3.3 Hz), 3.02 (1H, ddddd, J=5.7, 7.4, 7.6, 7.6, 8.0 Hz, 25-H), 2.40—2.29 (2H, m, 4 and 9-H), 2.16 (1H, ddd, J=12.6, 12.6, 7.5 Hz, 5-H), 1.97—1.90 (2H, m, 6 and 17-H), 1.87—1.69 (4H, m, 6, 15, 16 and 23-H), 1.63—1.48 (6H, m), 1.43—1.19 (6H, m), 1.16—1.05 (2H, m), 1.02 (3H, d, J=6.6 Hz, 21-H), 0.94 (1H, dd, J=3.5, 11.4 Hz), 0.90 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in acetone-d_6) δ: 84.6 (C-24, d), 73.2 (C-12, d), 72.4 (C-3, d), 71.4 (C-26, t), 68.4 (C-7, d), 61.5 (C-27, t), 47.8 (C-17, d), 47.3 (C-13, s), 43.1 (C-14, d), 42.8 (C-5, d), 41.1 (C-8, d), 40.9 (C-4, t), 40.7 (C-25, d), 36.6 (C-20, d), 36.6 (C-1, t), 36.0 (C-6, t), 35.7 (C-10, s), 32.0 (C-22, t), 31.6 (C-2, t), 29.8 (C-11, t), 29.4 (C-23, t), 28.6 (C-16, t), 27.7 (C-9, d), 24.1 (C-15, t), 23.4 (C-19, q), 18.1 (C-21, q), 13.1 (C-18, q).

Compound **4**: Colorless needles, mp 199—200 °C, $[\alpha]_D^{25}$ 28.9° (c=0.6, MeOH). *Anal.* Calcd for $C_{27}H_{46}O_5 \cdot 1/2H_2O$: C, 70.59; H, 10.45. Found: C, 70.36; H, 10.40. High-resolution MS: 432.3237 (error +0.2 mMU) for $C_{27}H_{44}O_4$ ($C_{27}H_{46}O_5 - H_2O$). IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2875, 1640, 1470, 1380, 1080, 1040, 1010, 980, 950, 920, 860. ^1H-NMR (in pyridine-d_5) δ: 6.22 (1H, d, J=5.0 Hz), 5.70 (1H, d, J=4.0 Hz), 5.36 (1H, d, J=4.0 Hz), 5.30 (1H, d, J=3.0 Hz), 5.01 (1H, dd, J=6.1, 6.1 Hz, 26-H_A), 4.88 (1H, dd, J=5.7, 7.9 Hz, 27-H_A), 4.78 (1H, dd, J=5.7, 7.9 Hz, 27-H_B), 4.70 (1H, dd, J=6.1, 6.1 Hz, 26-H_B), 4.30—4.26 (1H, m,

12-H), 4.12—4.08 (1H, m, 7-H), 4.08—4.03 (1H, m, 24-H), 3.77—3.71 (1H, m, 3-H), 3.16 (1H, ddddd, J=6.1, 6.1, 7.9, 7.9, 8.1 Hz, 25-H), 3.11 (1H, ddd, J=11.5, 11.7, 13.2 Hz, 4-H), 2.92 (1H, ddd, J=4.5, 12.5, 12.5 Hz, 9-H), 2.75 (1H, ddd, J=7.2, 12.3, 12.3 Hz, 5-H), 2.37 (1H, dd, J=9.6, 9.6 Hz, 17-H), 2.14—1.96 (3H, m), 1.96—1.76 (6H, m), 1.72—1.30 (9H, m), 1.27—1.18 (1H, m), 1.24 (3H, d, J=6.2 Hz, 21-H), 1.07 (1H, ddd, J=4.2, 13.4, 13.4 Hz, 1-H), 1.01 (3H, s, 19-H), 0.84 (3H, s, 18-H). ^{13}C-NMR (in pyridine-d_5) δ: 74.7 (C-26, t), 73.9 (C-27, t), 72.6 (C-24, d), 72.5 (C-12, d), 71.9 (C-3, d), 67.7 (C-7, d), 47.4 (C-13, s), 46.9 (C-17, d), 42.7 (C-14, d), 42.6 (C-5, d), 41.9 (C-25, d), 41.0 (C-4, t), 40.7 (C-8, d), 36.3 (C-1, t), 36.2 (C-20, d), 35.9 (C-6, t), 35.4 (C-10, s), 32.3 (C-22, t), 32.0 (C-23, t), 31.7 (C-2, t), 29.7 (C-11, t), 28.3 (C-16, t), 27.4 (C-9, d), 23.8 (C-15, t), 23.2 (C-19, q), 17.9 (C-21, q), 13.1 (C-18, q).

Alkaline Degradation of 2 with Potassium Hydroxide Vigorous alkaline hydrolysis of **2** (15 mg), in the manner described above, furnished compound **6** (9.0 mg). Recrystallization of compound **6** from aqueous EtOH gave 8.4 mg of colorless needles (mp 228—230 °C) (lit. 228—231 °C).[2] Compound **6** has the following physical properties.

Compound **6**: Colorless needles, $[\alpha]_D^{25}$ 39.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{27}H_{46}O_5 \cdot H_2O$: C, 69.23; H, 10.26. Found: C, 69.18; H, 9.93. High-resolution MS: 432.3210 (error −2.6 mMU) for $C_{27}H_{44}O_4$ ($C_{27}H_{46}O_5 - H_2O$). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1480, 1380, 1080, 1050, 1020, 980, 960, 920, 860. ^{1}H-NMR (in acetone-d_6) δ: 4.46 (1H, dd, J=6.2, 8.1 Hz, 26-H$_A$), 4.41 (1H, ddd, J=6.1, 6.1, 6.1 Hz, 24-H), 4.32 (1H, dd, J=6.2, 6.2 Hz, 26-H$_B$), 3.99—3.95 (1H, m, 12-H), 3.83—3.79 (1H, m, 7-H), 3.76 (1H, dd, J=6.0, 6.2 Hz, 27-H$_A$), 3.72 (1H, dd, J=6.2, 6.2 Hz, 27-H$_B$), 3.38—3.31 (2H, m), 3.27 (1H, d, J=4.3 Hz), 3.06 (1H, d, J=3.4 Hz), 2.76 (1H, ddddd, J=6.0, 6.1, 6.2, 6.2, 8.1 Hz, 25-H), 2.40—2.29 (2H, m), 2.17 (1H, ddd, J=7.6, 11.9, 11.9 Hz), 2.08—2.04 (2H, m), 1.97—1.92 (2H, m), 1.85—1.69 (6H, m), 1.64—1.48 (4H, m, 20, 22, 22 and 23-H), 1.45—1.32 (4H, m), 1.30—1.21 (1H, m), 1.17—1.05 (2H, m), 1.03 (3H, d, J=6.2 Hz, 21-H), 0.97—0.93 (1H, m), 0.92 (3H, s, 19-H), 0.71 (3H, s, 18-H). ^{13}C-NMR (in acetone-d_6) δ: 85.9 (C-24, d), 73.2 (C-12, d), 72.4 (C-3, d), 70.5 (C-26, t), 68.4 (C-7, d), 64.1 (C-27, t), 47.8 (C-17, d), 47.3 (C-13, s), 44.2 (C-25, d), 43.1 (C-14, d), 42.8 (C-5, d), 41.1 (C-8, d), 40.9 (C-4, t), 36.6 (C-20, d), 36.6 (C-1, t), 36.0 (C-10, s), 35.7 (C-6, t), 34.6 (C-23, t), 31.6 (C-22, t), 31.0 (C-2, t), 29.5 (C-11, t), 28.6 (C-16, t), 27.7 (C-9, d), 24.1 (C-15, t), 23.4 (C-19, q), 18.2 (C-21, q), 13.1 (C-18, q). From these data and by direct comparison with an authentic sample, compound **6** was identified as (24*R*,25*S*)-(+)-24,26-epoxy-5β-cholestane-3α,7α,12α,27-tetrol (anhydroscymnol).[5]

Bile Constituents of Sharks Material: Gallbladders, obtained from sharks, *Lamna ditropis* (*ca.* 20 kg) collected in July 1989 off the coast of Sanriku, Japan, *Chlamydoselachus anguineus* GERMAN (*ca.* 10 kg) collected May 1987 at Suruga Bay, Shizuoka Prefecture, Japan, *Glyphis glaucus* (*ca.* 8 kg) collected in November 1986 near Suruga Bay, and *Rhizoprionodon acutus* (*ca.* 8 kg) collected in November 1986 near Suruga Bay, Shizuoka Prefecture, Japan, were each homogenized and the homogenate was freeze-dried. These materials were used as a source.

Analysis of the Bile of Sharks: According to the procedure described above for sodium scymnol sulfate, the MeOH eluate of each shark was prepared and analyzed by HPLC. Each bile was shown by HPLC to contain **2**, which was identified by comparison of its spectral data with those of authentic **2**.

References and Notes

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