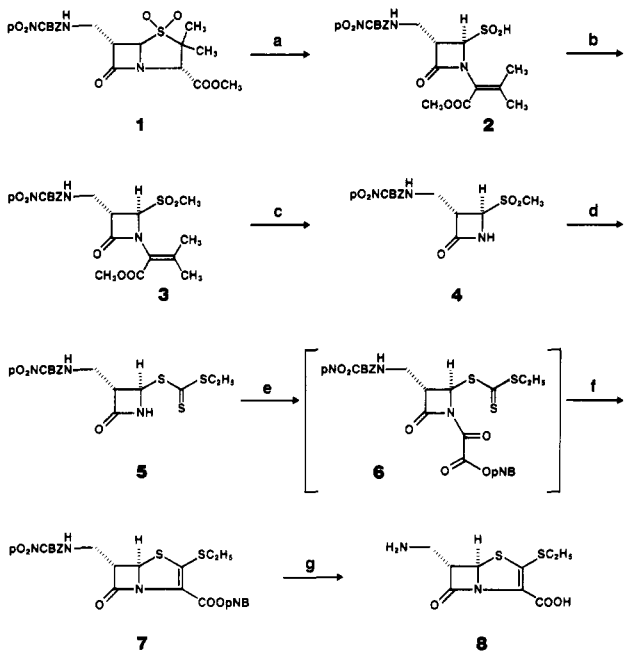
Scheme I[a]



[a] (a) DBU, CH_2Cl_2; (b) n-Bu_4NHSO_4, CH_3I; (c) $KMnO_4$, $NaIO_4$, pH 7; (d) $C_2H_5SC(S)S^-$, n-Bu_4NBr, CS_2; (e) ClC(O)C(O)O-p-NB, DIPEA, CH_2Cl_2; (f) $(C_2H_5O)_3P$, $CHCl_3$, Δ ; (g) H_2, 10% Pd on Celite; p-NO_2Cbz=$O_2NC_6H_4CH_2OC(O)$—; p-NB=$CH_2C_6H_4NO_2$; DIPEA=$C_2H_5N(i\text{-}C_3H_7)_2$.

oxycarbonyl)amino]methyl]penemcarboxylate (7). A solution of 0.680 g (1.64 mmol) of methyl 3-[[(4-nitrobenzyloxycarbonyl)amino]methyl]-2-oxoazetidin-4-yl trithiocarbonate and 0.797 g (3.27 mmol) of chloro 4-nitrobenzyl oxalate in 30 mL of ethanol-free $CHCl_3$ was treated with 0.327 g (3.27 mmol) of $CaCO_3$ and cooled to 3 °C. Then a solution of 0.422 g (3.27 mmol) of diisopropylethylamine in a few milliliters of ethanol-free $CHCl_3$ was added dropwise such that the temperature of the reaction mixture remained below 5 °C. This mixture was stirred for 30 min in an ice bath and then poured into ice water. The $CHCl_3$ layer was separated and combined with one $CHCl_3$ wash of the aqueous layer, and the mixture was dried over Na_2SO_4 and filtered.

The resulting solution containing the intermediate oxalamide was heated to reflux, and a solution of 0.815 g (4.91 mmol) of distilled $P(OEt)_3$ in 25 mL of $CHCl_3$ was added dropwise over a period of 4 h. Then the reaction mixture was heated 6 h more and was stirred at room temperature for 12 h. The solution was evaporated, and the residues were taken up in EtOAc from which the product crystallized, giving 215 mg (23%) of crystals: mp 205–207 °C; IR(KBr) 5.59, 5.92 μm; NMR (DMSO-d_6) δ 1.32 (3 H, t, J = 7 Hz) 3.03 (2 H, d of q, J = 7 Hz), 3.52 (2 H, br t), 4.01 (1 H, t, J = 7 Hz), 5.20 (2 H, s), 5.37 (2 H, AB q, J = 8, 25.4 Hz), 5.69 (1 H, d, J = 1.5 Hz), 7.81 (1 H, t, J = 7 Hz), 7.90 (4 H, d of d, J = 8, 158 Hz), 7.95 (4 H, d of d, J = 8, 130 Hz); $[\alpha]^{22}_D$ +92.7° (c 1.0, DMSO). Anal. Calcd for $C_{24}H_{22}N_4O_9S_2$: C, 50.17; H, 3.86; N, 9.75. Found: C, 50.16; H, 3.93; N, 9.47.

(5*R*,6*S*)-6-(Aminomethyl)-2-(ethylthio)penemcarboxylic Acid Sodium Salt. A solution of 235 mg of (5*R*,6*S*)-4-(nitrobenzyl) 2-(ethylthio)-6-[[(4-nitrobenzyloxycarbonyl)amino]methyl]penemcarboxylate in 25 mL of THF was diluted with 25 mL of water, and then 35 mg of 10% Pd on Celite was added. This mixture was hydrogenated at 50 psi for 1 h, and then a second portion of catalyst was added followed by hydrogenation for 30 min. The mixture was filtered through Celite, and the THF was evaporated. The aqueous residue was extracted two times with EtOAc and with ether, and the solution was then filtered through a Millipore filter and freeze-dried to afford 45 mg of a tan solid. This material was not pure[13], but the presence of the desired product was indicated by spectral data: IR(KBr) 5.65, 6.19 μm; NMR (D_2O) δ 1.37 (3 H, t, J = 7 Hz), 3.00 (2 H, d of q, J = 7 Hz), 3.36 (2 H, d of d, J = 1.5, 7 Hz), 4.10 (1 H, t, J = 6 Hz), 5.70 (1 H, d, J = 1.5 Hz); $[\alpha]^{22}_D$ +15.6° (c 1.0, H_2O).

Synthesis of a New Heterocycle: *cis*-4b,5,6,10b,11,12-Hexahydro-5,12-diazachrysene

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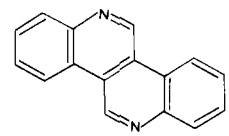
The 6,12-diazachrysene chromophore is a structural moiety found in degradation products such as calycanine (**6**).[1] The reported syntheses[2] of calycanine being rather



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lengthy and low yielding, we investigated a straightforward approach to diazachrysenes. Retrosynthetic analysis indicated that they could be obtained via Beckmann rearrangement of bis oxime **3a**. The required intermediates were prepared by methanesulfonic acid (MSA) cyclization of diphenylsuccinic acid (**1**).[3] Complete stereoconversion was observed during the ring-closure procedure. Once one ring is formed, only the cis-oriented carboxy and phenyl groups can further cyclize to give the diketo derivative **2**. The latter was converted into a mixture of isomeric oximes **3a** upon treatment with $HONH_2$·HCl/Na_2CO_3 (Scheme I).

The Beckmann rearrangement of **3a** to bis lactam **4** was then attempted. Treatment of the bis oxime under various Beckmann rearrangement conditions (PPA,[4] HMPT,[5] PPSE,[6] $POCl_3$/pyridine[7]) resulted either in extensive decomposition or fragmentation. Additionally a two-stage procedure[8] involving formation of a bromonitroso derivative followed by its reaction with triphenylphosphine did not lead to formation of any isolable product. The corresponding tosylate **3b** was also unreactive possibly because of its poor solubility in the acidic reaction media.

These disappointing results prompted us to examine the reductive Beckmann rearrangement.[9] When the bis oxime **3a** was allowed to react with a large excess of diisobutyl aluminium hydride (DIBAH) a basic compound was obtained in 23% yield. Surprisingly the ^1H NMR spectrum was consistent with a nonsymmetric structure. An ABX and a benzylic AB systems were present. Furthermore the angular protons appeared as a doublet and a doublet of triplet (X part of the ABX). Their small coupling constant (4.8 Hz) indicated that the ring junction was cis. Confirmation of the structure **5** was obtained by ^{13}C NMR. The chemical shift of C-1a occured approximately 10 ppm downfield from that of C-6a.

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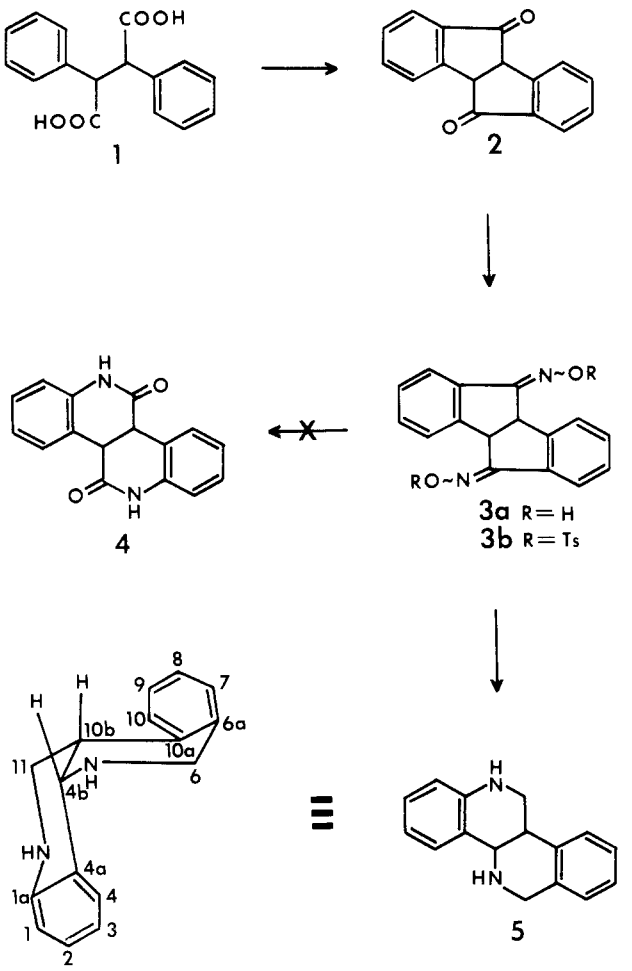
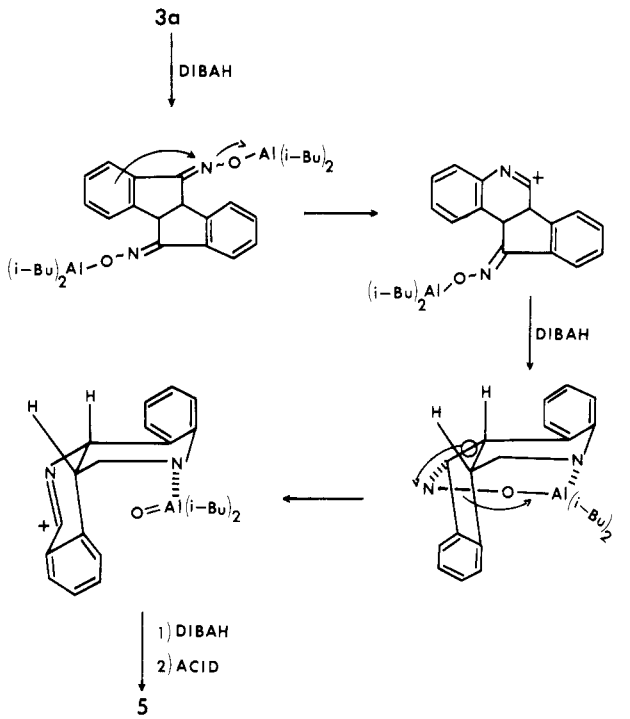
Scheme I



Scheme II



This approach made the new fused heterocycle *cis*-5,12-azachrysene **5** readily available in an overall yield of 11% over three steps.

The unexpected formation of **5** from bis oxime **3a** can be explained by stepwise reaction of the oxime groups (Scheme II). The mechanism of the Beckman rearrangement consists essentially of formation of electron-deficient nitrogen atoms with simultaneous migration of groups anti to departing hydroxy groups. The amphoteric aluminium reagent serves as a Lewis acid for the induction of the rearrangement and as a reductive agent to form the amine. As can be expected, a phenyl group migrates first. Then the remaining oxime aluminoxy can adapt a syn conformation relative to the second phenyl group as a consequence of intramolecular complexation. A methine group migration is then being observed.

Experimental Section

NMR spectra were measured on a Bruker Spectrospin WH-360 (360 MHz) spectrometer. Melting points were determined on a Büchi SMP-20 apparatus and are not corrected. Mass spectra were also determined for all the compounds and were consistent with the proposed structures. The reactions were routinely carried out under a dry argon atmosphere. Flash chromatography with Merck 230–400 mesh silica gel was used for purifications.

***cis*-4b,5,9b,10-Tetrahydroindeno[2,1-*a*]indene-5,10-dione (2).** A mixture of methanesulfonic acid (302 mL, 4.7 mole) and P_2O_5 (30.2 g, 213 mmol) was heated at 150 °C until a clear solution was obtained. To this **1** (18.9 g, 70.0 mmol) was added, and the resulting mixture was stirred at 150 °C. After 1 h of reaction the clear solution was cooled, diluted with H_2O (200 mL), and extracted with EtOAc. The organic layer was washed with 2 N $NaHCO_3$, dried over $MgSO_4$, and concentrated to a solid. Recrystallization (Et_2O–EtOAc) afforded pure **2** (8.2 g, 50% as white solid): mp 206–207 °C (lit.[10] mp 203–205 °C); ^1H NMR ($CDCl_3$) δ 4.40 (s, 2 H), 7.43 (t, J = 8 Hz, 2 H), 7.70 (m, 4 H), 7.92 (d, J = 8 Hz, 2 H).

***cis*-4b,5,9b,10-Tetrahydroindeno[2,1-*a*]indene-5,10-dione Dioxime (3a).** A mixture of diketone **2** (2.0 g, 8.5 mmol), Na_2CO_3 (1.0 g, 9.5 mmol), and $HONH_2$·HCl (1.8 g, 26.0 mmol) in MeOH (40 mL) and H_2O (2 mL) was refluxed for 1.5 h. After the solution was cooled to room temperature, H_2O (50 mL) was added and the solution extracted with EtOAc. The organic phase was dried over $MgSO_4$ and concentrated to half its volume. The resulting white crystals (1.6 g, 73%) were filtered, and the filtrate was evaporated to dryness. The brown solid obtained was recrystallized (MeOH–EtOAc) to give additional **3a** (0.6 g, 26%) as white needles: mp >300 °C dec; ^1H NMR (DMSO-d_6) δ 4.54 (d, J = 6 Hz, 2 H), 5.04 (s, 2 H), 5.11 (d, J = 6 Hz, 2 H), 7.22–7.32 (m, 8 H), 11.42 (s, 1 H), OH), 11.48 (s, 1 H, OH), 11.53 (s, 1 H, OH).

Anal. Calcd for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.51; N, 10.70. Found: C, 72.69; H, 4.53; N, 10.73.

***cis*-4b,5,9b,10-Tetrahydroindeno[2,1-*a*]indene-5,10-dione Bis[*O*-(*p*-tolylsulfonyl)oxime] (3b).** At −20 °C a solution of oxime **3a** (1.0 g, 3.8 mmol) in dry pyridine (10 mL) was slowly treated with tosyl chloride (1.7 g, 9.1 mmol). After 1 h at −20 °C the reaction was kept overnight in a refrigerator (4 °C). The solution obtained was then poured on ice, and the resulting precipitate was filtered and washed with ether to afford **3b** (1.9 g, 90%): mp 197–198 °C dec; ^1H NMR (DMSO-d_6) δ 2.42 (s, 3 H), 2.44 (s, 3 H), 2.49 (s, 3 H), 4.75 (d, J = 6 Hz, 2 H), 5.23 (s, 2 H), 5.31 (d, J = 6 Hz, 2 H), 7.65–8.20 (m, 16 H).

Anal. Calcd for $C_{30}H_{25}S_2N_2O_6$: C, 62.91; H, 4.24; N, 4.93; S, 11.22. Found: C, 62.52; H, 4.40; N, 4.88; S, 11.21.

***cis*-4b,5,6,10b,11,12-Hexahydro-5,12-diazachrysene (5).** A suspension of **3a** (1.0 g, 3.8 mmol) in dry CH_2Cl_2 (50 mL) was treated with DIBAH (15.6 mL of a 1.2 M solution in hexane) at −20 °C. After the mixture was stirred for 1 h at −20 °C the starting material dissolved. Stirring was continued for 3 h at 0 °C, and afterwards 2 N HCl (50 mL) was added. The acidic water layer was separated, neutralized with 2 N NaOH, and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, concentrated to an oil, and chromatographed on silica gel (EtOAc–hexane, 2:1) to give **5** (0.2 g, 23%) as colorless oil: ^1H NMR ($CDCl_3$) δ 3.98 (d, J = 4.8 Hz, 1 H, H-4b), 3.96 and 4.38 (AB, J = 14.8 Hz, 2 H, H-6 and H-6′), 3.03 (dt, J = 7.6 Hz, 1 H, H-10b), 3.29 and 3.37 (AB part of ABX, J_{AX} = 4.7 Hz, J_{BX} = 7.6 Hz, J_{AB} = 24 Hz, 2

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H, H-11 and H-11'), 6.55–7.65 (m, 8 H); ^{13}C NMR δ 144.6 (C-1a), 114.6 (C-1), 129.5 (C-2), 117.5 (C-3), 131.2 (C-4), 122.5 (C-4a), 53.0 (C-4b), 48.5 (C-6), 135.8 (C-6a), 126.0 (C-7), 126.3 (C-8), 126.5 (C-9), 128.4 (C-10), 136.1 (C-10a), 44.7 (C-11), 36.7 (C-10b).

Anal. Calcd for $C_{16}H_{16}N_2$: C, 81.35; H, 6.78; N, 11.86. Found: C, 81.31; H, 6.80; N, 11.84.

Acknowledgment. We thank Mrs. T. Zardin and Mr. H. R. Loosli for helpful discussion concerning the NMR data and Miss C. Fritz for the supply of large quantities of intermediates.

Toxic Trichothecenes from *Fusarium sporotrichioides* (MC-72083)

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The trichothecene mycotoxins are responsible for alimentary toxic aleukia (ATA), vomiting, weight loss, skin inflammation, and death in humans and farm animals from ingestion of *Fusarium* infected grains.[1] Recent efforts in our laboratories aimed at understanding the role secondary metabolites produced by *F. sporotrichioides* (MC-72083) play in the above noted illnesses have led to new relatively nontoxic trichothecenes.[2-4] We now report the structures and bioactivities of two new toxic metabolites: FS-2 and trichotriol, as well as 8-oxo-DAS, trichodiol, and 3-hydroxytrichothecene, a decomposition product of trichotriol.

F. sporotrichioides (MC-72083) was cultured and subjected to chromatographic workup as previously reported.[2,5]

8-Oxo-DAS (*diacetoxyscirpenol*) (**1**), 2 mg (30 ppb), $C_{19}H_{24}O_8$ (*m/z* 380.147, calcd 380.146), exhibited the following fragments in its electron-impact mass spectrum (EI-MS): *m/z* 380 (relative intensity) M^+ (10), 320 (1), 247 (5), 173 (10), 121 (20), and 43 (100). The IR (film) spectrum of **1** indicated that hydroxyl (3439, 1040, cm^{-1}), carbonyl (1739, 1734 cm^{-1}), and ether (1236 cm^{-1}) functionalities were pesent.[6] From the ^1H NMR (300 MHz, $CDCl_3$) data, four methyl groups at δ 0.82 (s, C-14), 1.84 (br s, 16-H, coupled to δ 6.62, 10-H), 2.00 (s, OAc), and 2.16 (s, OAc) were observed, together with a vinyl proton at 6.62 (dd, *J* = 2.9, 5.9 Hz, 10-H) and the characteristic splitting pattern of the 12,13-epoxide methylene protons at δ 2.82 and 3.10 (d, *J* = 3.9 Hz). Complete proton assignments were made by using COSY[7] (Table I) and are in accord with structure **1** as depicted. Comparison of the ^1H NMR data of **1** with that of a synthetic sample[8] confirmed the structure as depicted. The UV (λ_{max} 226 nm, ϵ_{max} 6100, ACN) indicated the presence of an α,β-unsaturated ketone which was suggested from the ^1H NMR and is supported by the ^{13}C NMR (75 MHz, $CDCl_3$) data (δ 136.8 d, C-10; 138.3, s, C-9). The presence of an unsaturated ketone at δ 196.6 (s, C-8) and two acetates at δ 172.6 (s) and 170.1 (s) were also indicated by ^{13}C NMR. Comparison of the NMR data of **1** with that of the closely related diacetoxyscirpenol[9] (DAS) **2** allowed ^{13}C NMR assignments to be made (Table I). This is the first report of naturally occurring 8-oxo-DAS.

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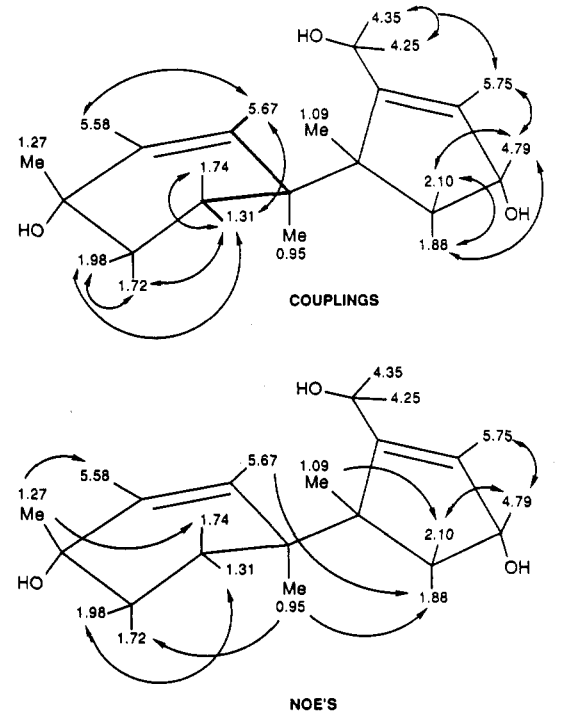

Figure 1. Couplings obtained from COSY and NOE's obtained from DNOES are shown for FS-2 (**3**). The W coupling between 11-H (δ 5.67) and 7-H (1.31) is depicted in bold lines.

FS-2 (**3**), 2 mg (30 ppb), $C_{15}H_{24}O_3$ (*m/z* 252.173, calcd 252.173), exhibited peaks at *m/z* 253 ($M^+ + 1$; 2), 235 ($M^+ - OH$; 50), 127 (85), 125 (90), 109 (127 – H_2O; 100), 107 (125 – H_2O, 85) in the CIMS. The large peaks at *m/z* 125 and 127 correspond to cleavage of the C-5/C-6 bond in **3** and gives rise to resonance stabilized fragments.

The UV (λ_{max} 194.5, ϵ_{max} 12 000, ACN) data was appropriate for two ene $\pi \rightarrow \pi^*$ transitions.[10] From inspection of the ^1H NMR spectrum (300 MHz, $CDCl_3$) three methyl singlets (δ 0.95, 1.09, 1.27), a methylene next to oxygen (δ 4.23, 4.35, J_{AB} = 14.5 Hz), two vinyl protons on an isolated *cis*-1,2-disubstituted ene (δ 5.58, d, *J* = 10.2 Hz; 5.67, dd, *J* = 1.7, 10.2 Hz), and a vinyl proton (δ 5.75, dd, *J* = 1.5, 3.3 Hz) coupled to a methine next to oxygen (δ 4.79, m) were observed. COSY[7] and DNOES (difference nuclear Overhauser effect spectroscopy) allowed completion of the structure and facilitated proton assignments (Table I). Significant couplings (COSY) and NOE's (DNOES) are shown in Figure 1, confirming the relative stereochemistry of **3** as depicted. The absolute stereochemistry is assumed

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