benzene to give 17-oxa-5α-androstan-3β-ol 3-acetate (13 mg). The mass spectral fragmentation pattern and the intensity of each fragment of this oxasteroid was entirely identical with those obtained from oxasteroid **17** prepared with ordinary mercury(II) oxide.

(b) Preparation of a Formate Corresponding to Formate 18 by Irradiation of the Hypoiodite of 4,4-Dimethyl-5α-cholestan-3β-ol in the Presence of Mercury(II) Oxide-^{18}O and Iodine and Its Transformation into Oxasteroid 24. Steroidal alcohol **20** (89 mg) in benzene (11 mL) containing mercury(II) oxide-^{18}O (140 mg) and iodine (163 mg) in a Pyrex vessel was treated as in the case of the reaction with ordinary mercury oxide. The resulting formate **21** (28 mg) was transformed into oxasteroid **24** (14 mg). The mass spectrum of **24** showed that the fragmentation pattern and the intensity of each fragment was identical with those obtained from oxasteroid **24** prepared with ordinary mercury(II) oxide.

Registry No. **4**, 1225-43-0; **5**, 83625-92-7; **6**, 83625-93-8; **7**, 91712-60-6; **8**, 91712-61-7; **9**, 83679-49-6; **10**, 83679-50-9; **11**, 54482-41-6; **12**, 83679-51-0; **14**, 3090-70-8; **15**, 83625-94-9; **16**, 83625-95-0; **17**, 83625-96-1; **17**-ol, 83632-45-5; **18**, 83679-52-1; **19**, 83625-97-2; **20**, 2550-84-7; **21**, 85382-31-6; **22**, 91712-62-8; **23**, 91712-63-9; **24**, 83626-02-2; **25**, 2542-65-6; **26**, 91712-64-0; **27**, 91712-65-1; **28**, 91712-66-2; **29**, 83626-01-1; **30**, 15064-05-8; **31**, 91712-67-3; **32**, 91712-68-4; **33**, 91712-69-5; **34**, 91712-70-8; **48**, 19043-45-9; **49**, 80-97-7; **51**, 566-88-1; **52**, 1251-59-8; **53**, 1251-58-7; **54**, 91712-71-9; **55**, 91712-72-0; **56**, 91712-73-1; **57**, 91796-74-6; **58**, 85382-32-7; **59**, 49540-03-6; **60**, 85382-33-8; **61**, 91712-74-2; **62**, 91712-75-3; **63**, 72489-61-3; **64**, 280-11-5; **65**, 284-20-8; **66**, 91712-76-4; **67**, 91712-77-5; $Hg^{18}O$, 91712-78-6; 2,2-dimethyl-cholest-4-en-3-one, 17305-84-9; 2,2-dimethyl-5α-cholestan-3-one, 2542-57-6; cyclopentanal, 96-41-3; cyclohexanol, 108-93-0; cyclohexanone, 108-94-1; cycloheptanol, 502-41-0; cycloheptanone, 502-42-1; cyclooctanol, 696-71-9; cyclooctanone, 502-49-8.

Structural Studies of the Mycotoxin Verrucosidin

Maya Ganguli,† Leo T. Burka,‡ and Thomas M. Harris*

Department of Chemistry and Center in Molecular Toxicology, Vanderbilt University, Nashville, Tennessee 37235

Received February 14, 1984

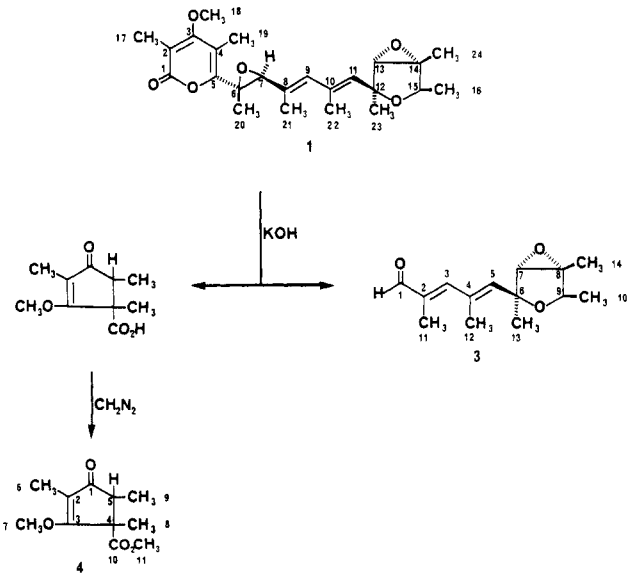
Structure 1 has been established for verrucosidin, a neurotoxin isolated from *Penicillium verrucosum* var. *cyclopium*. A novel fragmentation in base yields aldehyde **3** and a rearranged cyclopentenone carboxylic acid. The structure of the methyl ester **4** of this acid has been established by independent synthesis. A mechanism is proposed for the formation of the cyclopentenone ring of **4** from the epoxy α-pyrone of **1**.

In a recent report we reported the structure of the tremorgenic mycotoxin, verrucosidin (**1**), which was isolated from *Penicillium verrucosum* var. *cyclopium*.[1] The isolation and identification of verrucosidin were prompted by local reports of neurotoxicoses in cattle caused by hay; investigations of this hay revealed the presence of two tremorgen-producing fungi, one of which is *P. verrucosum* var. *cyclopium*, the other has not yet been identified.[2]

Verrucosidin, a potent neurotoxin (LD_{50} in mice, 4 mg/kg, i.p.) which causes sustained tremoring in experimental animals, was isolated from the hay samples. Larger quantities have been obtained by growing the fungus on a potato–milk–sucrose medium and extracting the air-dried fungal pad with ether. Silica gel chromatography followed by recrystallization from ether gave **1** as colorless plates, mp 90–91 °C, $[a]^{26}_D$ +92.4° (*c* 0.25, methanol). The empirical formula $C_{24}H_{32}O_6$ was determined by mass spectrometry (*m/z* 416) and by elemental analysis.

The ^1H NMR spectrum was rather simple in appearance, consisting of nine methyl resonances, only one of which showed substantial coupling, and five signals integrating for one hydrogen each, two of which were vinyl hydrogens and the other three methines. Only one of the methines showed substantial coupling (to the methyl group); the other one-proton signals were somewhat broadened but no useful coupling information could be obtained at 90 MHz. Twenty-four resonances were observed in the ^{13}C NMR spectrum. The chemical shifts of the five downfield signals suggested an α-pyrone unit. This

Scheme I



hypothesis was corroborated by the IR (ν_{max} 1700 cm^{-1}) and UV (λ_{max} 294 nm, ε 13 000 and 241 nm, ε 21 000) spectra.

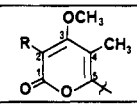
† Division of Physical and Life Sciences, Fayetteville State University, Fayetteville, NC 28301.

‡ National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

(1) Burka, L. T.; Ganguli, M.; Wilson, B. J. *J. Chem. Soc., Chem. Commun.* **1983**, 544.
(2) Wilson, B. J.; Byerly, C. S.; Burka, L. T. *J. Am. Vet. Med. Assoc.* **1981**, *179*, 480.
(3) Steyn, P. S.; Vleggar, R.; Wessels, P. L.; Woudenberg, M. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2175.
(4) Niwa, M.; Endo, T.; Ogiso, S.; Furukawa, H.; Yamamura, S. *Chem. Lett.* **1981**, 1285.
(5) Kruger, G. J.; Steyn, P. S.; Vleggar, R. *J. Chem. Soc., Chem. Commun.* **1979**, 441.

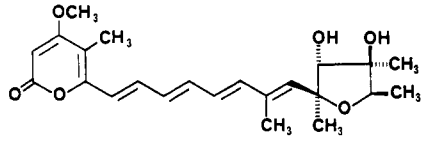
0022-3263/84/1949-3762$01.50/0

Table I. ^{13}C NMR Chemical Shift Data (ppm) for the Pyrone Moiety of Verrucosidin (1) and the Other Related Mycotoxins



	C-1	C-2	C-3	C-4	C-5	4-CH_3	3-OCH_3
verucosidin[1] (R = CH_3)	164.5 or 167.5	110.0 or 110.6	167.5 or 164.5	110.6 or 110.0	155.6	8.9	60.0
citreoviridin[3] (R = H)	163.7	88.1	170.4	107.5	154.2	8.6	56.0
citreoviridinol[4] (R = H)	163.7	88.4	170.5	107.6	154.3	8.8	56.1
asteltoxin[5] (R = H)	161.9	88.6	170.3	107.9	154.1	8.1	56.0
aurovertin B[6,7] (R = H)	163.6	88.7	169.8	108.0	154.3	8.9	56.2

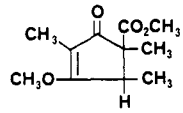
Comparison of the ^{13}C spectrum of **1** with those of citreoviridin (**2**) and related α-pyrone natural products



2

(Table I) reveals a close agreement of the chemical shifts for all but C-2, which is methylated in **1** but unsubstituted in the other compounds.

Hydrolysis of **1** by base (Scheme I) was attempted early in the study of the structure. As described in the earlier report, an aldehyde (**3**) containing all but one of the carbon atoms in the side chain of **1** was one product; a carboxylic acid of unknown structure was the other. The carboxylic acid was isolated by careful acidification of the alkaline hydrolysis mixture (after removal of aldehyde **3** by extraction) followed by extraction into methylene chloride. Purification by HPLC yielded the acid as a solid, the UV spectrum (λ_{max} 253 nm, ϵ 11 500) of which suggested the presence of a β-alkoxy-α,β-unsaturated ketone. The ^1H NMR spectrum consisted of four methyl signals at δ 1.15, 1.45, 1.94, and 4.16, a methine quartet at δ 2.34 coupled to the methyl signal at 1.15, and a carboxylic acid proton signal at δ 8.45. The parent ion in the mass spectrum was at m/z 198. Treatment of the acid with diazomethane followed by chromatography on silica gel gave the ester as a colorless liquid, $[\alpha]^{25}_D$ +27.4° (*c* 0.27, $CDCl_3$), which had the molecular formula $C_{11}H_{16}O_4$. Capillary GLC revealed the presence of two apparently isomeric substances in an 8:1 ratio. The ^{13}C NMR spectrum of the major component contained signals for five methyl groups, two of which were attached to oxygen. Signals at 112.8 and 181.4 ppm were attributed to a vinyl ether moiety. Additional signals indicated a methine carbon (51.9), a quaternary carbon (54.8), a ketone (205.7), and an ester (172.7 ppm). 4-(Methoxycarbonyl)-3-methoxy-2,4,5-trimethylcyclopenten-2-one (**4**) seemed the most likely structure for the ester, although on the basis of spectra alone we could not rule out the 5-carbomethoxy isomer **5**. Compound **5**

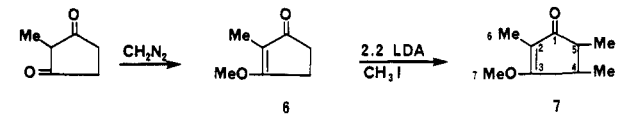


5

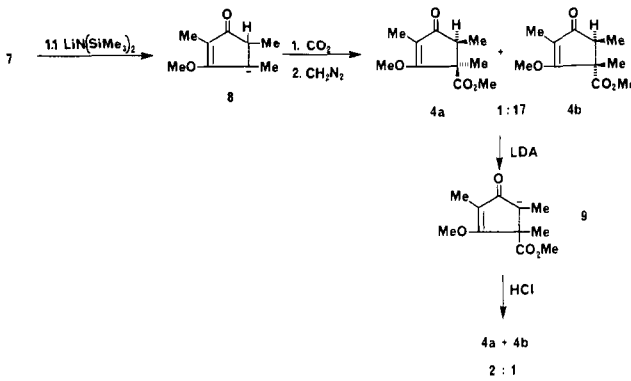
would, however, be expected to decarboxylate more readily than **4**. No information was available as to the relative configurations at C-4 and C-5.

In order to identify the carboxylic acid, we attempted regio- and stereoselective syntheses of **4** and **5**. Our strategy was based upon the observation that 3-alkoxycyclopentenones on treatment with lithium diisopropylamide form enolate anions by ionization of the 5 proton,[8] which is α to the carbonyl group, whereas lithium bis(trimethylsilyl)amide ionizes the 4 proton, which is γ to the carbonyl group.[9] 3-Methoxy-2-methylcyclopent-2-enone (**6**) was prepared in quantitative yield by treating 2-methyl-1,3-cyclopentanedione with excess diazomethane (Scheme II). The dianion of **6** was generated with 2.2 equiv of lithium diisopropylamide;[9] subsequent reaction with excess methyl iodide gave dimethyl derivative **7** as a 45:1 stereoisomeric mixture. A long-range coupling (^5J = 1.3 Hz) between the 6-methyl protons and the 4-methine proton was observed for the major form of **7**; similar coupling could be observed with **6**. No coupling was observed between the 6-methyl group and the proton at C-5. Assignments of the ^1H signals for the C-8 and C-9 methyl groups of **7** were made from shift reagent studies by using $Eu(fod)_3$ which complexes primarily with the keto group. Similar long-range coupling between the 6-CH_3 and 4-CH_2 protons of **6** has been observed by Cimarusti and Wolinsky[10] and was confirmed in the present work.

Scheme II



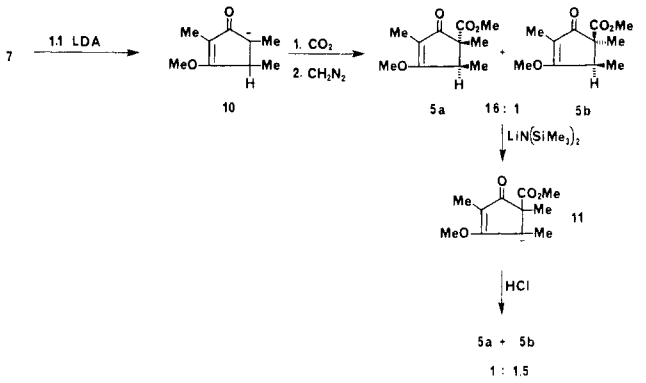
Scheme III



Treatment of ketone **7** with 1.1 equiv of lithium bis(trimethylsilyl)amide at −78 °C produced monoanion **8** which was treated with carbon dioxide (Scheme III). The resulting acid was esterified with diazomethane. Column chromatography yielded a mixture of esters **4a** and **4b** as a colorless liquid. Capillary GLC indicated that they were present in a 1:17 ratio. The long-range coupling seen in the ^1H spectra of compounds **6** and **7** was not observed in **4b**, indicating that carboxylation had occurred as anticipated at C-4. The relative configurations of **4a** and **4b** were

(6) Mulheirn, L.; Beechey, R. B.; Leworthy, D. L. *J. Chem. Soc., Chem. Commun.* **1974**, 874.
(7) Steyn, P. S.; Vleggar, R.; Wessels, P. L. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1298.
(8) Quesada, M. L.; Schlessinger, R. H.; Parsons, W. H. *J. Org. Chem.* **1978**, *43*, 3968; *J. Am. Chem. Soc.* **1980**, *102*, 889. Stork, G.; Danheiser, R. L. *J. Org. Chem.* **1973**, *38*, 1775.
(9) Koreeda, M.; Liang, Y.; Akagi, H. *J. Chem. Soc., Chem. Commun.* **1979**, 449. Koreeda, M.; Mislankar, S. G. *J. Am. Chem. Soc.* **1983**, *105*, 7203.
(10) Cimarusti, C. M.; Wolinsky, J. *J. Org. Chem.* **1966**, *31*, 4118.

Scheme IV



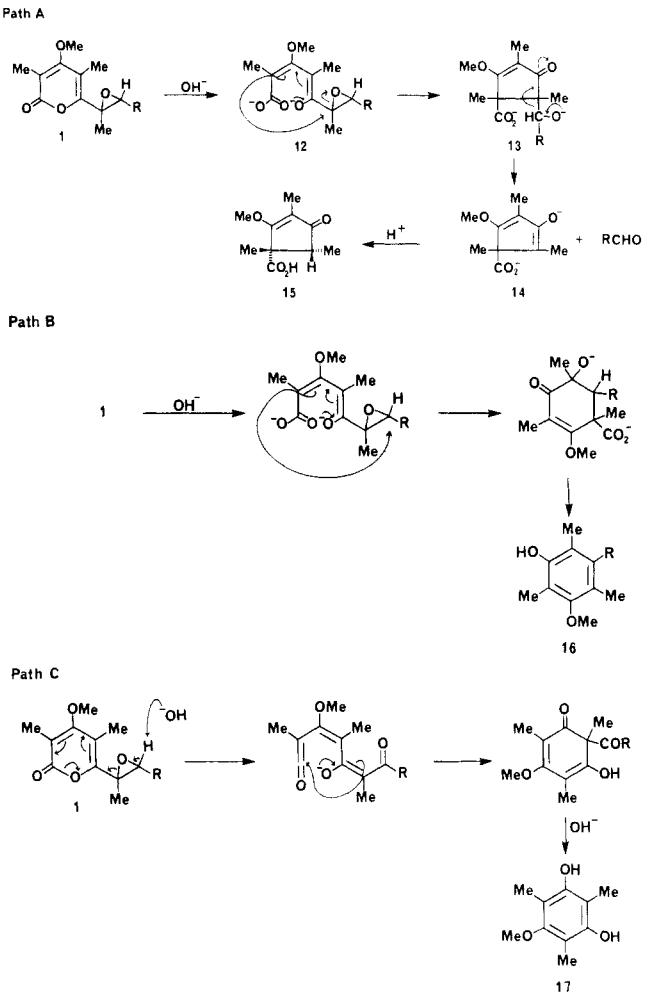
assigned by treatment of the mixture with 1.1 equiv of lithium diisopropylamide followed by kinetic protonation of anion **9** with dilute hydrochloric acid. The process gave a 2:1 mixture of **4a** and **4b**. Preparative separation of **4a** from **4b** by liquid chromatography was not possible in either case, but having the two mixtures, one rich in **4a** and the other in **4b**, made it possible to assign some of the signals in the spectra, most significantly in the ^1H spectrum the 5-CH group at δ 2.30 in **4a** vs. 2.75 in **4b** and in the ^{13}C spectrum the C-8 methyl group at δ 21.3 in **4a** vs. 17.2 in **4b**. The 8-CH_3 signal in the ^{13}C NMR spectrum of **4b** is shifted upfield relative to that of **4a** by steric interaction with the C-9 methyl group. The ^1H and ^{13}C NMR spectra of **4a** were identical with those of the major ester derived from verrucosidin. Moreover, comparison by capillary GLC showed that the major ester from verrucosidin was identical with **4a** and the minor one with **4b**.

Carboxylation of the 5 anion of ketone **7** was also investigated (Scheme IV). Treatment of **7** with 1.1 equiv of lithium diisopropylamide gave monoanion **10** which was treated with carbon dioxide at 0 °C to produce a carboxylic acid which was esterified with diazomethane. Whereas the acid derived from verrucosidin (and from carboxylation of the 4 anion of **7**) was relatively stable, the acid obtained from the 5 anion was thermally unstable and immediate treatment with diazomethane was necessary to avoid decarboxylation. Again a mixture of two methyl esters was obtained. Capillary GLC indicated a 16:1 mixture of **5a** and **5b**. In the ^1H NMR spectrum (of the major component) a long-range coupling (^5J = 1.2 Hz) could be observed between 6-CH_3 and 4-CH as expected for isomers of **5**. The mixture was enriched in **5b** by conversion to anion **11** by treatment with lithium bis(trimethylsilyl)amide followed by quenching with dilute hydrochloric acid. Capillary GLC indicated that the recovered material was a 1:1.5 mixture of **5a** and **5b**. A preparative separation of the two esters was not obtained. In their ^{13}C NMR spectra, the C-9 signal was ~5 ppm higher field in **5a** than in **5b**, providing the basis for their relative assignments.

The stereochemistry of carboxylation of anions **8** and **10** and of protonation of anions **9** and **11** can be rationalized on steric grounds. Anions **8** and **10** show strong preference for carboxylation on the face bearing a proton rather than a methyl group at the adjacent position, giving better than 10:1 selectivity in both cases. Less selectivity is observed in protonation of anions **9** and **11** where one face bears a methyl group and the other a carbomethoxy group; in both cases protonation on the side bearing the methyl group is slightly favored. Likewise bis-methylation of the dianion **6** should give primarily the trans form of **7**.

A mechanism for the fragmentation of verrucosidin by base can be proposed (see Scheme V). The initial step

Scheme V



is envisaged as a hydrolysis of the pyrone ring. Hydrolytic cleavage of α-pyrones is a well-precedented reaction.[11] The resulting dienolate anion **12** then attacks the proximal epoxide to give cyclopentenone **13**. Retroaldol cleavage of **13** gives enolate anion **14** which undergoes protonation predominantly (8:1 ratio) on the less hindered methyl face to give mainly **15** (the carboxylic acid corresponding to ester **4a**). It is noteworthy that the cleavage–recyclization process with **1** occurs by attack at the more hindered position of the epoxide (path A); the alternative process (path B) would yield a six-membered ring which could give **16** by dehydration and decarboxylation. Path A is favored over B by the more favorable trajectory of nucleophilic attack on the epoxide and conforms with Baldwin's rules,[12] i.e., an exo-5 tetrahedral cyclization is favored over an endo-6 and is supported by precedents.[13] Yet another possible reaction of **1** with base could have been an attack on the epoxide proton to give the enolate anion of the isomeric ketone. Anions of analogous keto pyrones undergo a ring-opening and recyclization, probably via a ketene intermediate (path C), to give benzenoid products.[14] In the present case **17** would have been formed; no trace of either **16** or **17** was observed.

The tremorgenic mycotoxins which have been hitherto identified have all contained at least one nitrogen atom (usually an indole) in their structures.[15] Thus **1** is unusual

(11) For a review, see: Harris, T. M.; Harris, C. M. *Tetrahedron* **1977**, *33*, 2159.
(12) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734.
(13) Stork, G.; Cama, L. D.; Coulson, D. R. *J. Am. Chem. Soc.* **1974**, *96*, 5268. Stork, G.; Cohen, J. S. *Ibid.* **1974**, *96*, 5270.
(14) Harris, T. M.; Wachter, M. P. *Tetrahedron* **1970**, *26*, 5255.

in this respect. The compounds in Table I are all neurotoxins and are close structural analogues of 1, the most important difference probably being their lack of the epoxides. Citreoviridin (2) is the only one that has been studied extensively;[16] the symptoms of toxicity of citreoviridin in experimental animals have been described as including paralysis of limbs, convulsions, and respiratory arrest but no mention is made of sustained trembling.[16,17]

Experimental Section

Nuclear magnetic resonance spectra were recorded on JEOL MH-100 and FX-90Q spectrometers using tetramethylsilane as internal standard in $CDCl_3$ unless otherwise indicated. Infrared spectra were obtained with Perkin-Elmer Model 727 spectrophotometer. Ultraviolet spectra were recorded on Cary 14 and Cary 210 spectrophotometers. Optical rotations were measured at room temperature (23–28 °C) on an Autopole III polarimeter. Gas–liquid chromatography was carried out on a Shimadzu GC-MINI-2 chromatograph. A 30 m × 0.25 mm SE-30 capillary column was used as 150 °C. A flame ionization detector was used. Column, thin-layer, and preparative-layer chromatography were performed on Merck silica gel 60. Mass spectra were obtained on an LKB type 9000 or VG-Micromass 7070F spectrometer. Elemental analyses were done by Galbraith Laboratories, Knoxville, TN. All melting points were determined on a Thomas-Hoover melting points apparatus and are uncorrected. All organometallic reactions were carried out under nitrogen in oven-dried glassware. Solvents were carefully dried before use.

Verrucosidin (1). Instant potatoes (20 g), non-fat dry milk (30 g), sucrose (20 g), and gentamycin sulfate (100 mg) were placed in a baking pan and suspended in 1 L of deionized water. The pan was tightly covered with aluminum foil and autoclaved (121 °C, 1 h × 2) and then inoculated with a suspension of mycelium of *P. verrucosum* var. *cyclopium* in sterilized water containing a drop or two of Tween 80. The incubation mixture was allowed to stand at room temperature (24–25 °C) for 2 weeks. A crude preparation containing toxin was obtained by extracting the chopped, air-dried mat with ether in a Soxhlet apparatus. The extract residue, dissolved in a minimum amount of ethyl acetate–hexane (1:3) was placed on a short column of silica gel and eluted with ethyl acetate–hexane (1:3). Chromatography of the partially purified toxin using ethyl acetate-hexane (1:2) gave 50–100 mg of 1 which could be further purified by recrystallization from ether to give colorless plates, mp 90–91 °C; $[\alpha]^{26}_D$ +92.4° (*c* 0.25, MeOH); UV λ_{max}^{MeOH} 294 (ϵ 13 000) and 241 nm (ϵ 21 000); IR ($CDCl_3$) 1575, 1640, 1700, and 2970 cm^{-1}; ^1H NMR δ 1.21 (3 H, d, J = 7 Hz, 16-CH_3), 1.42, 1.44, and 1.48 (total of 9 H, 3 × s, 20-, 23-, and 24-CH_3), 1.93, 1.98, 2.05, and 2.05 (total of 12 H, 4 × s, 17-, 19-, 21-, and 22-CH_3), 3.45 and 3.50 (total of 2 H, 2 × s, 7- and 13-CH), 3.85 (3 H, s, 18-OCH_3), 4.12 (1 H, q, J = 7 Hz, 15-CH), 5.50 and 5.88 (total of 2 H, 2 × br s, 9- and 11-CH); ^{13}C NMR δ 8.9, 10.0, 13.5, 15.0, 15.2, 18.2, 18.5, and 21.6 (C-16, 17, 19, 20, 21, 22, 23, 24), 60.0 (C-18), 60.3 (C-6), 64.4 (C-7), 67.1 (C-14), 67.1 (C-13), 76.4 (C-15), 79.7 (C-12), 110.0 and 110.6 (C-2 and 4), 127.7 (C-8 or 10), 131.1, 132.7 (C-9 and 11), 134.2 (C-10 or 8), 155.6 (C-5), 164.5, 167.5 (C-1 and C-3); MS (electron impact), m/z (relative intensity) 416 ($M^{+\cdot}$, 0.1), 127 (100), 97 (99); MS (chemical ionization) (CH_4), m/z (relative intensity) 417 ($M + H^+$, 100), 399 (73), 345 (28), 249 (33), 197 (42), 183 (43), 127 (90).

Anal. Calcd for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.38; H, 7.83.

Hydrolysis of Verrucosidin. Verrucosidin (290 mg, 0.7 mmol) was dissolved in 3 mL of methanol, aqueous potassium hydroxide (1 mL, 1 M) was added, and additional methanol was added (0.5 mL) to give a homogeneous solution. After 22–24 h at room temperature, the reaction mixture was diluted with water (4 mL) and extracted with dichloromethane. The organic solution was dried (Na_2SO_4) and concentrated; the residue (140 mg) was purified by column chromatography (ethyl acetate–hexane 1:4) to give 60 mg of 3 as a yellow oil: $[\alpha]^{27}_D$ −23.5° (*c* 0.4, MeOH); λ_{max}^{MeOH} 277 (ϵ 11 800); IR ($CDCl_3$) 1670 and 1610 cm^{-1}; ^1H NMR δ 1.19 (3 H, d, J = 7 Hz, 10-CH_3), 1.45, 1.48, 1.95, and 2.16 (total of 12 H 4 × s, 11-, 12-, 13-, and 14-CH_3), 3.44 (1 H, s, 7-CH), 4.15 (1 H, q, J = 7 Hz, 9-CH), 5.91 (1 H, s, 5-CH), 6.68 (1 H, s, 3-CH) and 9.41 (1 H, s, 1-CH); ^{13}C NMR δ 10.8, 13.8, 17.7, 18.9, 21.5 (C-10, 11, 12, 13, and 14), 67.2 (C-7), 67.5 (C-8), 76.8 (C-9), 80.0 (C-6), 135.2 (C-2), 137.1 (C-4), 140.2 (C-5), 154.1 (C-3) and 195.8 (C-1); MS, m/z (relative intensity) 236 ($M^{+\cdot}$, 1.0), 221 (17), 207 (15), 189 (29), 137 (33), 125 (27), 123 (50), 109 (100). In addition 70 mg of 1 (25%) were recovered.

The aqueous layer from the extraction was carefully acidified to pH 2 with 1 N sulfuric acid and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and concentrated to give crude 4-carboxy-3-methoxy-2,4,5-trimethylcyclopent-2-enone (110 mg). Part of this material was purified by HPLC (C-18 reverse phase, MeOH–H_2O–HCO_2H, 30:70:1) to yield the pure acid 15 as a solid: IR ($CDCl_3$) 1730, 1620, 1455, and 1380 cm^{-1}; ^1H NMR δ 1.15 (3 H, d, J = 7 Hz, 9-CH_3), 1.45 (3 H, s, 8-CH)3 1.94 (3 H, s, 6-CH_3), 2.34 (1 H, q, J = 7 Hz, 5-CH), 4.16 (3 H, s, 7-CH_3), and 8.45 (1 H, br s, 10-CO_2H); ^{13}C NMR δ 8.1 (6-CH_3), 11.1 (9-CH_3), 21.0 (8-CH_3), 50.9 (5-CH), 54.7 (4-C), 59.3 (7-CH_3), 113.0 (2-C), 176.7 (10-CO), 181.6 (3-C), and 206.0 (1-CO); MS (electron impact), m/z (relative intensity) 198 ($M^{+\cdot}$, 1.0), 154 (49), 139 (100).

The remaining crude acid (60 mg) was treated with excess ethereal diazomethane. The ether was removed and a residue was purified by column chromatography using ethyl acetate–hexane (1:4) as eluent to give 4 (mainly 4a) as a colorless liquid: $[\alpha]^{25}_D$ +27.4° (*c* 0.27, $CDCl_3$); λ_{max}^{MeOH} 248 nm (ϵ 11 600); ^1H NMR (4a) δ 1.06 (3 H, d, J = 7.5 Hz, 9-CH_3), 1.47 (3 H, s, 8-CH_3), 1.95 (3 H, s, 6-CH_3), 2.30 (1 H, d, J = 7.5 Hz, 5-CH), 3.68 (3 H, s, 11-CH_3), 4.1 (3 H, s, 7-CH_3); ^{13}C NMR (4a) δ 8.1 (6-CH_3), 11.0 (9-CH_3), 21.3 (8-CH_3), 51.2 (11-CH_3), 51.9 (5-CH), 54.8 (4-C), 59.1 (7-CH_3), 112.8 (2-C), 172.7 (10-CO), 181.4 (3-C), 205.7 (1-CO); exact mass measurement M_r calcd for $C_{11}H_{16}O_4$ 212.10485, found 212.1051; analysis by GLC indicated the material contained two components: 4a (t_r 12.6 min) and 4b (t_r 13.9 min) in a ratio of 8:1.

3-Methoxy-2-methylcyclopent-2-enone (6). 2-Methyl-1,3-cyclopentanedione (100 mg, 0.9 mmol) was treated with excess diazomethane in ether. The ether was removed to give colorless crystals of 6 (110 mg, 100%), mp 61–62 °C (lit.[8] mp 59.5–60 °C).

3-Methoxy-2,4,5-trimethylcyclopent-2-enone (7). A solution of 6 (1.26 g, 10 mmol) in 10 mL of THF was added slowly to 22 mmol of lithium diisopropylamide (prepared from 2.5 g (25 mmol) of diisopropylamine and 22 mmol of *n*-butyllithium in 100 mL of THF at −78 °C) at −78 °C. The mixture was stirred for an additional 30 min. A solution of methyl iodide (4.26 g, 3/ mmol) in THF (2 mL) was added dropwise. After 1 h at 0 °C, cold 3 N HCl was added, followed by ~70 mL of water. The mixture was extracted with ether. The ethereal layer was dried and concentrated to give a residue (1.63 g). Column chromatography using ethyl acetate–hexane (1:3) gave 7 (920 mg, 60%) as a yellow liquid. Capillary GLC indicated the material contained two components with t_r 5.57 and 6.63 min in a ratio of 45:1. NMR spectra of the major component are reported: ^1H NMR δ 1.16 (3 H, d, J = 6 Hz, 9-CH_3), 1.24 (3 H, d, J = 6 Hz, 8-CH_3), 1.79 (3 H, d, J = 1.3 Hz, 6-CH_3), 1.99 (1 H, m, 4-CH), 2.39 (1 H, m, 5-CH), and 4.08 (3 H, s, 7-CH_3); ^{13}C NMR δ 6.8 (C-6), 15.5 (C-8 or C-9), 17.5 (C-9 or C-8), 41.5 (C-4), 47.9 (C-5), 57.5 (C-7), 111.5 (C-2), 184.5 (C-3), and 207.2 (C-1).

4-(Methoxycarbonyl)-3-methoxy-2,4,5-trimethylcyclopent-2-enone (4). A solution of 7 (250 mg, 1.62 mmol) in THF (1 mL) was added slowly to 1.78 mmol of lithium bis(trimethylsilyl)amide (prepared from 1.8 mmol, 0.38 mL, of hexamethyldisilazane and 1.78 mmol of *n*-butyllithium in 10 mL of THF) at −78 °C. The mixture was stirred for 1 h. Subsequently carbon dioxide was passed through the solution for 20 min at −78 °C. The reaction mixture was allowed to warm to −10 °C over a period of 40 min and quenched with dilute hydrochloric acid. Water (5 mL) was added and the mixture was extracted with ether. The ether solution was washed with sodium bicarbonate solution; the sodium bicarbonate solution was cooled and acidified with

(15) Cole, R. J. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V., Hasseltine, C. W., Mehlman, M. A., Eds.; Pathotox publishers, Inc.: Park Forest South, IL, 1977; p 583.
(16) Veno, Y. In "Mycotoxins"; Purchase, I. F. H., Ed.; Elsevier: New York, 1974; p 283.
(17) Steyn, P. S. In "Mycotoxins in Human and Animal Health"; Rodricks, J. V., Hasseltine, C. W., Mehlman, M. A., Eds.; Pathotox Publishers, Inc.: Park Forest South, IL, 1977; p 421.

6 N HCl and the extracted with dichloromethane. the dichloromethane solution was dried and concentrated to give 160 mg of a solid acid, 140 mg of which was treated immediately with ethereal diazomethane. The methyl ester was purified by chromatography using ethyl acetate-hexane (1:4) as eluent to give 130 mg of **4** as a colorless liquid. Capillary GLC of the aterial revealed that **4a** and **4b** (t_r 12.6 and 13.9 min) were present in a ratio of 1:17; M_r calcd for $C_{11}H_{16}O_4$ 212.10485, found 212.1053; ^{1}H NMR (**4b**) δ 1.11 (3 H, d, J = 7.5 Hz, 9-CH_3), 1.31 (3 H, s, 8-CH_3), 1.93 (3 H, br s, 6-CH_3), 2.75 (1 H, q, J = 7.5 Hz, 5-CH), 3.71 (3 H, s, 11-CH_3), and 4.11 (3H, s, 7-CH_3); ^{13}C NMR (**4b**) δ 8.0 (C-6), 10.4 (C-9), 17.2 (C-8), 48.4 (C-5), 52.4 (C-11), 53.2 (C-4), 59.2 (C-7), 111.4 (C-2), 174.2 (C-10), 182.4 (C-3), and 206.4 (C-1).

The mixture of esters **4a,b** (50 mg, 0.24 mmol) was treated with lithium diisopropylamide (0.3 mmol) at -78 °C for 1 h. The reaction mixture was quenched with dilute hydrochloric acid and extracted with ether. The ether solution was dried and concentrated to give a colorless liquid which was revealed by capillary GLC to be a 2:1 mixture of **4a** and **4b**. Both ^{1}H and ^{13}C NMR spectra confirmed the presence of esters **4a** and **4b**; attempts to separate them on a preparative basis were unsuccessful.

5-(Methoxycarbonyl)-3-methoxy-2,4,5-trimethylcyclopent-2-enone (5). A solution of **7** (280 mg, 1.8 mmol) in THF (~2 mL) was added dropwise to a solution of lithium diisopropylamide (2 mmol) in 10 mL of THF at -78 °C. After 30 min, the mixture was warmed to 0 °C and kept at that temperature for 30 min. Carbon dioxide was passed through the mixture at 0 °C for 30 min. Workup as in the previous carboxylation afforded the crude acid (190 mg) which was found to undergo decarboxylation on standing. The acid was, therefore, immediately treated with diazomethane. Purification by chromatography gave 150 mg (39%) of **5** as an oil which solidified at -20 °C. Capillary GLC indicated the material was a mixture of **5a** and **5b** (t_r 15.0 and 14.2 min, respectively) in a ratio of 16:1; M_r calcd for $C_{11}H_{16}O_4$ 212.10485, found 212.1058; ^{1}H NMR (**5a**) δ 1.12 (3 H, d, J = 7 Hz, 8-CH_3), 1.30 (3 H, s, 9-CH_3), 1.86 (3 H, d, J = 1.2 Hz, 6-CH_3), 3.24 (1 H, d × d, J = 7.0 and 1.2 Hz, 4-CH), 3.72 (3 H, s, 11-CH_3), and 4.14 (3H, s, 7-CH_3); ^{13}C NMR (**5a**) δ 7.7 (C-6), 13.3 (C-8), 16.0 (C-9), 41.7 (C-4), 52.5 (C-11), 57.2 (C-5), 58.4 (C-7), 110.2 (C-2), 173.0 (C-10), 185.8 (C-3), and 202.7 (C-1).

The **5a,b** mixture was treated with lithium bis(trimethylsilyl)amide (0.3 mmol) at -78 °C for 1 h, quenched with dilute HCl, and extracted with ether. The residue from the ether solution was a colorless oil which capillary GLC showed to be a 1:1.5 mixture of **5a** and **5b**. Attempts to separate the esters preparatively were unsuccessful.

Acknowledgment. This research was supported by Grants ES 00267 and ES 00569 from the U.S. Public Health Service to Vanderbilt University. We thank Professor B. J. Wilson for his encouragement and support and for generous supplies of verrucosidin and Ms. J. Doran for technical assistance. The high-resolution mass spectrometer was purchased with a grant (GM-27557) from the U.S. Public Health Service.

Registry No. **1**, 88389-71-3; **3**, 88367-23-1; **4a**, 91366-57-3; **4b**, 91366-59-5; **5**, 91366-60-8; **5b**, 91366-58-4; **6**, 3883-56-5; **7**, 91384-67-7; **8**, 91366-61-9; **9**, 91384-68-8; **10**, 91366-62-0; **11**, 91384-69-9; **15**, 91366-63-1; 2-methyl-1,3-cyclopentanedione, 765-69-5.

Synthetic Anthracyclinones. 25.[1] An Improved Route to 8-Demethoxyaranciamycinone and Synthesis of the α-L-Daunosamine Glycosides

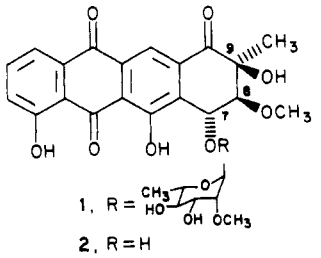
Karsten Krohn* and Erwin Broser

Institute of Organic Chemistry, Technische Universität Braunschweig, Schleinitzstrasse, D-3300 Braunschweig, West Germany

Received December 29, 1983

An improved regioselective synthesis for the anthracyclinone precursor **12** is reported using a combination of Diels-Alder and Marschalk reactions. The solvolysis of the benzylic bromides **19/20** with water mainly yields the 7,9-*trans*-diol 8-demethoxyaranciamycinone (**22**) whereas the treatment with sodium hydroxide affords the 7,9-*cis*-diol **21**. The α-L-daunosamine glycosides **27-30** are prepared and their absolute configurations are determined by ^{1}H NMR spectroscopy.

The anthracycline antibiotic aranciamycin (**1**) was isolated from *Streptomyces echinatus* 14 years ago.[2] However, the absolute configuration of **1** has only been determined recently by Sheldrick and Zeeck using X-ray analysis[3] and CD measurements.[4] In contrast to most other anthracyclinones,[5,6] aranciamycinone has an oxo group at C-10, an inverse configuration at C-9, a 7,9-trans configuration of the hydroxy groups, and an additional methoxy group at C-8.



Some time ago we published a synthesis of 8-demethoxyarancia mycinone (**22**)[7] starting from chrysophanol methyl ether (**12**). The preparation of **12** involved a methylation step, where expensive silver oxide had to be used, and, in addition, the yield of **12** was decreased by an elimination reaction. The new procedure is operationally simpler and better adopted for scaled up preparations of **12**, which is also a common precursor of other naturally

(1) Synthetic Anthracyclinones. 24. See: Krohn, K.; Sarstedt, B. *Angew. Chem.* **1983**, *95*, 897; *Angew. Chem. Suppl.* **1983**, 1267; *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 875.
(2) Keller-Schierlein, W.; Sauerbier, U.; Vogler, U.; Zähner, H. *Helv. Chim. Acta* **1970**, *53*, 779.
(3) Sheldrick, G. M.; Zeeck, A., manuscript in preparation.
(4) Zeeck, A., personal communication.
(5) Brockmann, H. *Fortschr. Chem. Org. Naturst.* **1963**, *21*, 121.
(6) Thomson, R. H. "*Naturally Occurring Quinones*"; Academic Press: London, New York, 1971; p 536.
(7) Krohn, K.; Broser, E. *Liebigs Ann. Chem.* **1982**, 1907.

0022-3263/84/1949-3766$01.50/0