69.5 °C; IR (Nujol) 3220, 1670 cm^{-1}; NMR ($CDCl_3$) δ 0.7–1.8 (m, 34 H), 2.2 (br t, 2 H), 3.6–4.5 (m, 5 H).

Anal. Calcd for $C_{21}H_{41}NO_2$: C, 74.28; 12.17; N, 4.12. Found: C, 74.08; H, 12.16; N, 4.06.

Phosphate Triester 7. To a stirred mixture of alcohol **6** (1.1325 g, 3.34 mmol) and triethylamine (0.3374 g, 3.34 mmol) in 50 mL of dry benzene, cooled to 0 °C, was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.4744 g, 3.34 mmol) in 2 mL of benzene in one portion. The mixture was stirred at room temperature for 2 h. The precipitated triethylamine hydrochloride was filtered off and the filtrate evaporated in vacuo to give the phosphate triester **7** (1.48 g, 100%) as a white solid: NMR ($CDCl_3$) δ 0.7–1.7 (m, 33 H, 2.25 (br t, 2 H), 4.25 (m, 9 H). The compound was used for the next step directly.

Phosphorylcholine 8. Phosphate triester **7** (1.45 g) was transferred with 50 mL of dry acetonitrile into a pressure bottle and 1 mL of anhydrous trimethylamine was added to it. The pressure bottle was left in an oil bath at 65 °C overnight. Cooling and filtration of the precipitated product gave 1.31 g of the crude oxazolinephosphorylcholine **8** as a white solid: IR (Nujol 1665 cm^{-1}; NMR ($CDCl_3$) δ 0.7–1.7 (m, 33 H), 2.25 (br t, 2 H), 3.35 (s, 9 H), 3.4–4.2 (m, 9 H); R_f($CHCl_3$–MeOH–H_2O, 65:25:4) 0.28, R_f($CHCl_3$–MeOH–aqueous NH_3, 1:9:1) 0.33. The material was used without purification.

1-Stearoyl-2-deoxy-2-aminophosphatidylcholine Hydrochloride (9). A 334-mg sample of phosphorylcholine **8** was stirred with 7 mL of 0.1 N HCl (1 equiv) for 48 h. The water was then removed by coevaporation several times with ethanol to give 370 mg of crude amino phosphorylcholine **9** as a colorless solid: IR (Nujol) 3360 (br), 1740 cm^{-1}; NMR ($CDCl_3$ + CD_3OD) δ 0.8–1.6 (m, 33 H), 2.25 (br t, 2 H), 3.15 (s, 9 H), 3.2–4.2 (m, 13 H); R_f($CHCl_3$–MeOH–aqueous NH_3, 1:9:1) 0.27. This material was used without further treatment.

1-Stearoyl-2-(stearoylamino)-2-deoxyphosphatidylcholine (2). A mixture of aminophosphorylcholine hydrochloride **9** (334 mg, 0.6 mmol), stearic anhydride (49 mg, 0.9 mmol), and 4-(dimethylamino)pyridine (150 mg, 1.22 mmol) in 25 mL of chloroform was stirred in the dark for 48 h. The solvent was then removed in vacuo. The residue was dissolved in 25 mL of methanol–chloroform–water (5:4:1, v/v) and passed through a column of Rexyn 1-300 resin (75 mL). The resin was washed with two bed volumes of the same solvent. After removal of the solvent in vacuo, the residue was chromatographed on a Sephadex LH-20 column (65 × 1.3 cm) to give 260 mg (55%) of 2-*sn*-amidophosphatidylcholine **2** as a colorless solid: IR (Nujol) 3270 (br), 1735, 1650 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.7–1.65 (m, 66 H), 1.9–2.3 (m, 4 H), 3.10 (s, 9 H), 3.2–4.3 (m, 10 H); R_f($CHCl_3$–MeOH–water, 65:25:4) 0.69, R_f($CHCl_3$–MeOH–aqueous NH_3, 1:9:1) 0.30; $[\alpha]^{25}_D$ −9.11° (*c* 0.95, $CHCl_3$).

Anal. Calcd for $C_{44}H_{89}N_2O_7P \cdot H_2O$: C, 65.47; H, 11.36; N, 3.47; P, 3.84. Found: C, 65.01; H, 11.28; N, 3.45; P, 3.81.[24]

2-[[(Octyloxy)carbonyl]amino]deoxylecithin (10). A mixture of aminophosphorylcholine hydrochloride **9** (120 mg, 0.215 mmol), octyl chloroformate (60 mg, 0.31 mmol), and 4-(dimethylamino)pyridine (50 mg, 0.41 mmol) in 25 mL of chloroform was stirred in the dark for 48 h. The solvent was removed and the residue dissolved in 100 mL of $CHCl_3$–MeOH–water (4:5:1, v/v) and passed through a column of Rexyn 1-300 resin (75 mL). The resin was washed 3 times with a 100-mL portion of the same solvent. After removal of the solvent, the residual product was chromatographed over silica gel (5 g) to give the carbamoyl derivative as a waxy solid: 85 mg (58%); IR (Nujol) 3460 (br), 1710 (br) cm^{-1}; NMR ($CDCl_3$–CD_3OD) 0.7–1.7 (m, 48 H), 2.1 (br t, 2 H), 3.0 (s, 9 H), 3.1–4.3 (m, 12 H); R_f($CHCl_3$–MeOH–water, 65:25:4) 0.69, R_f($CHCl_3$–MeOH–aqueous NH_3, 1:9:1) 0.38.

Anal. Calcd for $C_{35}H_{71}N_2OP \cdot H_2O$: C, 60.32; H, 10.77; N, 4.02; P, 4.44. Found: C, 60.32; H, 10.75; N, 4.20; P, 4.40.

2-[[(Octadecylamino)carbonyl]amino]deoxylecithin (11). A mixture of aminophosphorylcholine hydrochloride **9** and 4-(dimethylamino)pyridine (55 mg, 0.45 mmol) in 30 mL of chloroform was stirred in the dark for 48 h. The solvent was removed in vacuo, and the residue was dissolved in 100 mL of $CHCl_3$–MeOH–water (4:5:1, v/v) and passed through a column of Rexyn 1-300 resin (75 mL). The resin was washed 3 times with a 75-mL portion of the same solvent mixture. After removal of the solvent, the residue was triturated with pentane (50 mL) followed by acetone (50 mL). The residual solid was then chromatographed over silica gel (5 g) to give urea derivative **11** as a colorless solid: 108 mg (60%); IR (Nujol) 3330, 1735, 1635 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.6–1.5 (m, 68 H), 2.1 (br t, 2 H), 2.95 (s, 9 H), 3.0–4.2 (m, 13 H); R_f($CHCl_3$–MeOH–water, 65:25:4) 0.69.

Anal. Calcd for $C_{45}H_{92}N_3O_7P \cdot H_2O$: C, 64.63; H, 11.09; N, 5.03; P, 3.70. Found: C, 65.02; H, 11.04; N, 5.12; P, 3.71.[24]

Acknowledgment. We thank Professor Edward A. Dennis of the University of California, San Diego, for the enzymological experiments. We are grateful to the Research Corporation, the American Heart Association, Greater Boston Massachusetts Division and American Heart Association Massachusetts Affiliate, Inc. (Grant No. 13-503-798), and the National Institutes of Health (AM 26165) for financial support.

Registry No. **2**, 81218-95-3; **4**, 74983-20-3; **5**, 81218-96-4; **6**, 81206-32-8; **7**, 81206-33-9; **8**, 81206-34-0; **9**, 81206-35-1; **10**, 81206-36-2; **11**, 81206-37-3; 1-cyanoheptadecane, 638-65-3; methyl D-serinate hydrochloride, 5874-57-7; 2-chloro-2-oxo-1,3,2-dioxaphospholane, 6609-64-9.

N-Hydroxytryptophan in the Synthesis of Natural Products Containing Oxidized Dioxopiperazines. An Approach to the Neoechinulin and Sporidesmin Series

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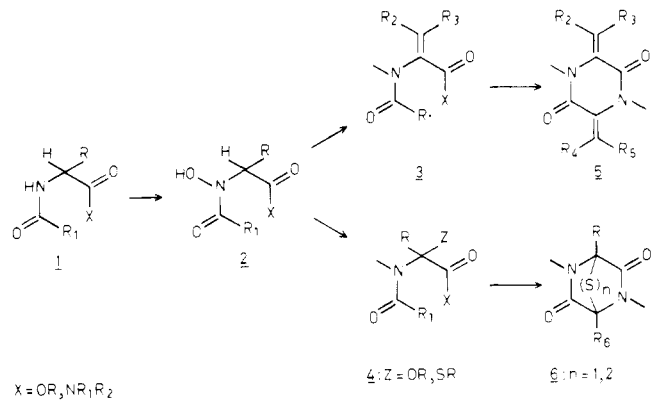
Received October 7, 1981

N-Hydroxytryptophan derivatives have been synthesized via two routes. In one procedure, the indole-3-pyruvate oximes **14b,c** were reduced with trimethylamine–borane in the presence of ethanolic HCl to give **18** (Scheme III); aminolysis of the latter afforded **19**. The second route features the reaction of *N*-methylindole (**20**) with the α-amino-β-bromopropionate **21** to yield **22** (Scheme IV). Subsequent aminolysis followed by trimethylamine–borane reduction gave **25**. Acylation with pyruvoyl chloride converted the compounds **19** and **25** into the dioxopiperazines **28** and **29**, as well as the tetracyclic product **30** and **31**, respectively (Scheme V). Compound **29** was transformed into the didehydrodioxopiperazine **32**, an analogue of neoechinulin B (**7a**). The structure of **30** was determined by X-ray crystallographic analysis.

In recent years α-amino acid derivatives with a functionality in addition to the amino and carboxy groups have been shown to be characteristic structural elements of several naturally occurring compounds. There are indi-

0022-3263/82/1947-2147$01.25/0

Scheme I



cations that several types of these nonprotein amino acids, viz., the *N*-hydroxy-α-amino acids **2**,[3] dehydroamino acids **3**,[4,5] and α-functionalized amino acids **4**[4,6] are interrelated with α-amino acids **1**. A possible biogenetic relationship between these compounds is presented in Scheme I.[4,6] We have shown that **2** deserves attention, not only as biosynthetic precursor but also as synthon for **3** and **4**; methods were developed for the synthesis of dehydroamino acid derivatives **3**[5a] as well as α-functionalized amino acid derivatives **4**[6a] by starting from O-alkylated or O-acylated derivatives of **2**. Both methods involve initial formation of the corresponding acylimines. They were also applied to the syntheses of simple didehydrodioxopiperazines **5**[5a] and sulfur-bridged dioxopiperazines **6**[7].

The structures **5** and **6**, with one amino acid residue being derived from tryptophan, are characteristic structural elements of the fungal metabolites which belong to the classes of the neoechinulins (**7**) and sporidesmins (**8**), respectively. Neoechinulin B and sporidesmin B have been synthesized by Kishi and co-workers;[8-10] neoechinulin A has been recently prepared by two groups in Japan.[11,12] We report another approach to **7** and **8** which features *N*-hydroxytryptophans.

Strategy

For the synthesis of the skeletons of neoechinulin B and sporidesmin B, i.e., compounds **7b** and **8b**, respectively, we chose the amide of *N*-hydroxytryptophan (**9**, Scheme II) as the starting material. For the construction of the dehydrodioxopiperazine ring of **10** we planned to apply the known[6a] pyruvoyl chloride reaction. Treatment of **10** with base in the *absence* of a nucleophile should then afford **7b** in a reaction sequence (**9** → **10** → **7b**) analogous to the conversion of **2** into **5**.[5a] On the other hand, treatment of **10** with base in the *presence* of a nucleophile X^- should afford **11**, which in turn might be transformed into the disulfide-bridged compound **8b**. This conversion combines the reaction sequence **2**–**6**[7] with the cyclization of tryptophan derivatives to the dihydropyrroloindoline system. The latter reaction has been amply studied[13-16] and has been used[9,10] for the syntheses of sporidesmin A and B.

Synthesis of *N*-Hydroxytryptophan Derivatives 9. Indole-3-pyruvic acid (**12**) was esterified and converted into the oxime ester **13** (Scheme III) in 66% yield[17] by treatment with *O*-benzylhydroxylamine hydrochloride in ethanol with *p*-toluenesulfonic acid as a catalyst. Aminolysis of **13** gave **15** in 93% yield. Methylation of the indole nitrogen of **13** failed on using NaH in monoglyme with subsequent addition of CH_3I in DMF,[19] but reaction with CH_3I–K_2CO_3 in DMF[20] gave **14c**, albeit in low yield (30%). The yield could be improved to 65% by Ag_2O catalysis.[21] Even better results were obtained by using KOH and CH_3I in Me_2SO, a procedure employed by Johnstone et al.[22] This method provided **14a** in 95% yield. Treatment of **14a** with diazomethane gave **14b** quantitatively. Aminolysis of **14b** and **14c** gave the amide **16** in yields of 86% and 90%, respectively.

Attempts to reduce the oximes of **15** and **16** failed (vide infra); however, as we knew from previous work[3a] that α-oximino *esters* are more easily reduced than α-oximino *amides*, we subjected the esters **13**, **14b**, and **14c** to reduction. Use of $(CH_3)_3N$–BH_3 in the presence of ethanolic HCl afforded the *N*-benzyloxy amino acid esters **17** and **18** in 50%, 49%, and 43% yields, respectively. Finally, aminolysis of **18** gave the desired *N*-hydroxytryptophan derivative **19** in 54% yield.

The preparation of **19** as depicted in Scheme III deserves some further comment. First, selective alkylation of the indole nitrogen in **17** to give **18** failed; as base treatment of the corresponding *N*-acyl-protected derivative of **17** would cause an elimination reaction, alkylation of the indole nitrogen had to precede the oxime reduction step. Second, all attempts to reduce selectively the oximes of **15** and **16** failed. In both cases reduction to the corresponding 2,3-dihydrotryptophan derivatives occurred.[23] This was not unexpected, since the reduction of indoles to 2,3-dihydroindoles with pyridine–borane has been reported.[24,25] It is surprising, however, that the esters **14b**

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(2) Department of Crystallography.
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(17) This reaction is considerably less efficient than the formation of other oxim esters,[18] which might be due to the acid lability of the indole nucleus.
(18) Herscheid, J. D. M.; Ottenheijm, H. C. J. *Tetrahedron Lett.* **1978**, 5143.
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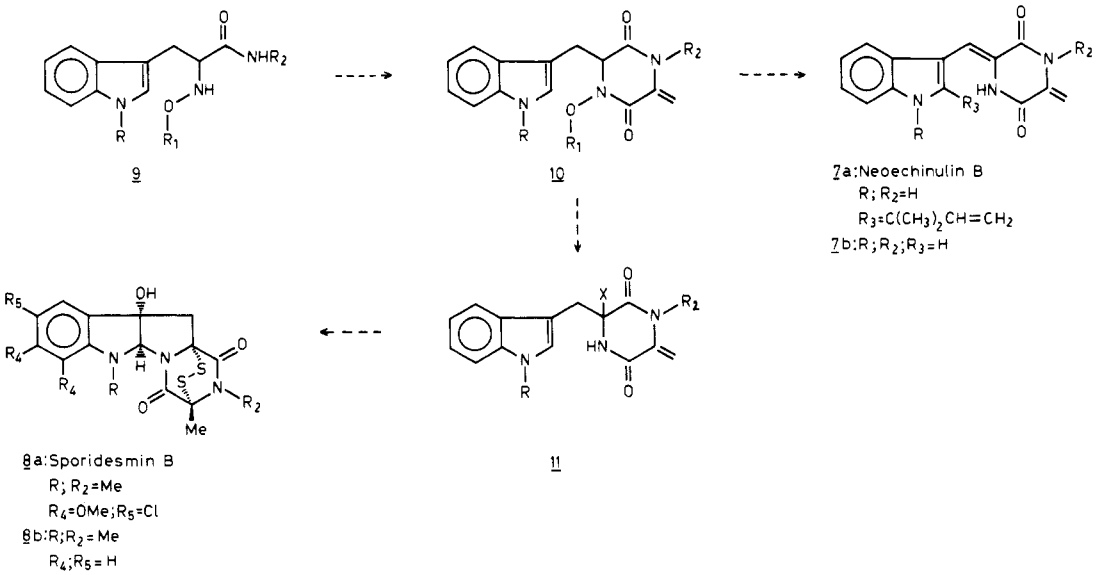
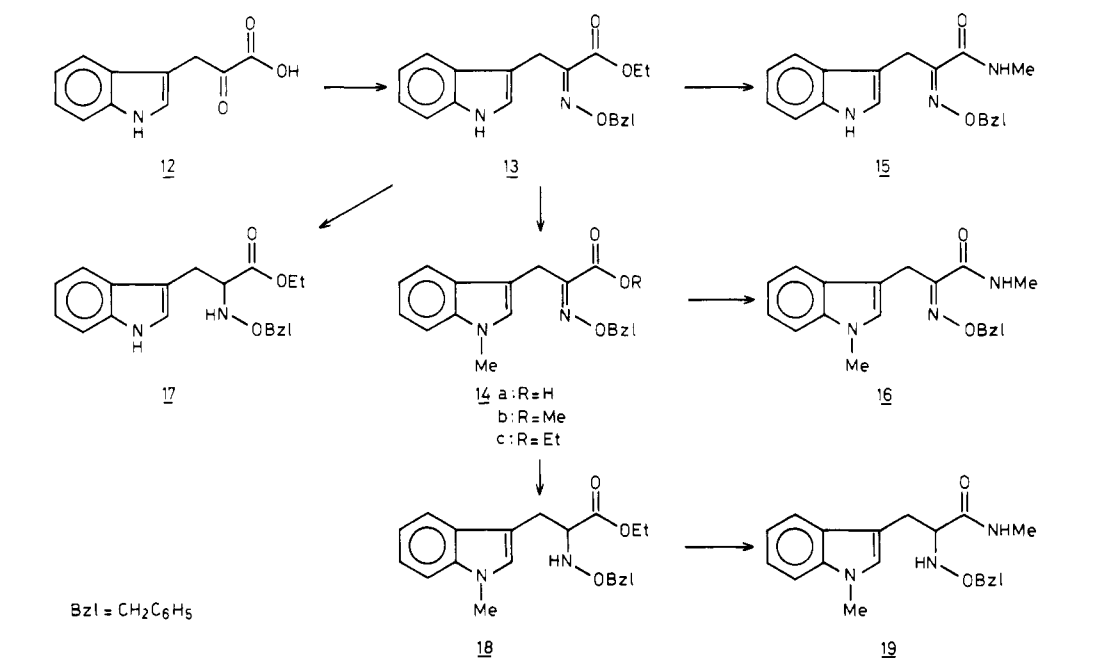
(22) Johnstone, R. A. W.; Rose, M. E. *Tetrahedron* **1979**, *35*, 2169.
(23) Concomitant oxime reduction was observed only with **15**, yielding (58%) the corresponding 2,3-dihydroindole derivative of **18**. From **16** was isolated the corresponding 2,3-dihydroindole derivative as the main product (55% yield). Attempts to oxidize these 2,3-dihydroindoles to the corresponding indoles with DCCQ were not successful.
(24) Kikugawa, Y. *J. Chem. Res., Synop.* **1977**, 212.
(25) Kikugawa, Y. *J. Chem. Res., Synop.* **1978**, 184.

Scheme II



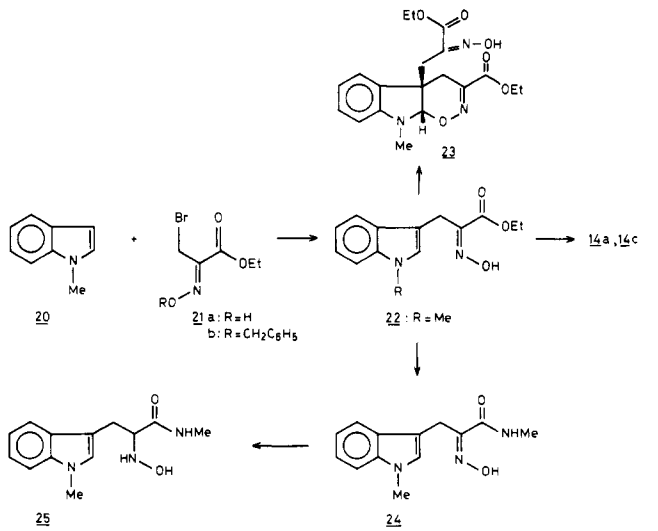
Scheme III



and **14c** can be converted into **18** in good yields without overreduction. The exact mechanism of oxime reductions with borane–amine complexes is still unknown; nevertheless, we are inclined to attribute the failure to reduce selectively the oximes of **15** and **16** to the competitive protonation of the amide functions. This slows down the oxime reduction rate, making the reduction of the indole nucleus a competitive reaction. This might not be the case with reduction of the oximes of **14b** and **14c**; once **18** has been formed, protonation of the (benzyloxy)amino function will occur. This positive charge will prevent the protonation of the indole ring, blocking its reduction.[26]

Consequently, the ester aminolysis (**18** → **19**) was performed subsequent to reduction of the oxime (**14** → **18**). Unfortunately, this order of operations has a drawback in that **18** suffers some replacement of its benzyloxy group

Scheme IV



(26) The same argument has been used by Kikugawa[25] to explain the failure to reduce tryptophan esters to the corresponding indoline derivatives, a reaction reported to proceed smoothly with *N*-acyltryptophane derivatives.

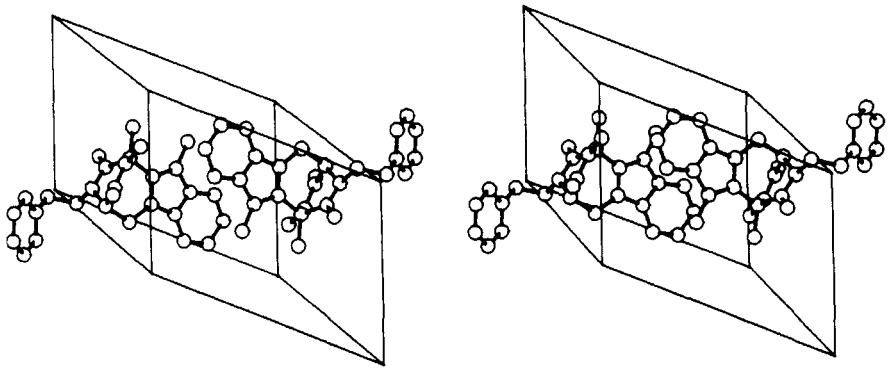


Figure 1. Stereoscopic view of **30** in the unit cell.

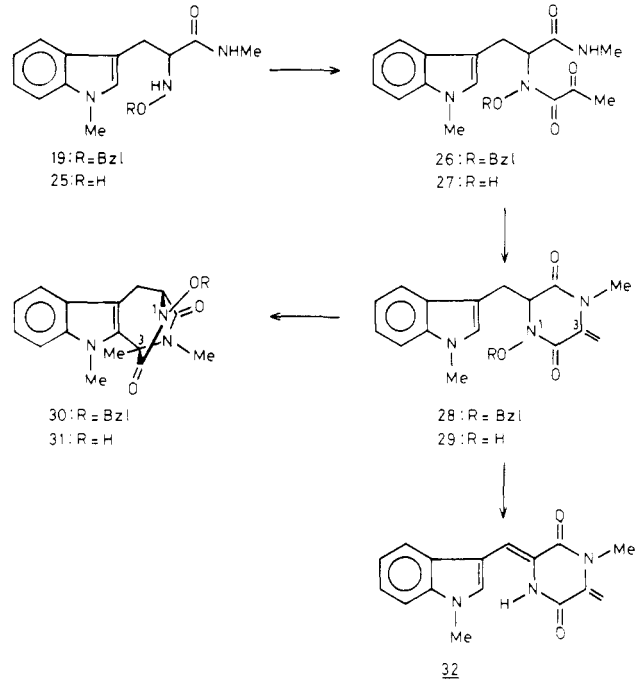
by $MeNH_2$, either in an S_N2[27] or in an elimination–addition process.[6a] This side reaction explains in part the moderate yield (54%) in the conversion of **18** into **19**.

Since the reaction sequence converting **12** into **19** involves several inefficient steps, an alternative approach was sought. A more versatile route to **9** (Scheme II), we thought, could possibly be devised by commencing with *N*-methylindole (**20**, Scheme IV). However, our attempts to convert **20** into **14c** by reaction with **21b**, using *n*-BuLi in THF or NaOH in a two-phase system, led to recovery of **20**. At that time, the reaction of **21a** with indole was reported;[28] the corresponding oximino ester **22** (R = H) was prepared in a reaction postulated as proceeding by the cycloaddition of a transient nitroso olefin to the indole C(2)–C(3) double bond, followed by base-catalyzed ring opening and rearomatiziation.[28] We found that *N*-methylindole (**20**) undergoes a similar reaction; treatment with Na_2CO_3 and **21a**, prepared quantitatively from ethyl bromopyruvate and hydroxylamine hydrochloride, gave **22** in 88% yield. Interestingly, in addition to **22**, a 2:1 adduct (**23**) was isolated in 5% yield. The formation of **23** could be suppressed by using a fourfold excess of **20**. The following experiment showed that **22** is a viable intermediate in the formation of **23**: treatment of **22** with **21a** and Na_2CO_3 gave (95% yield) the adduct **23**. The structure of **23** was inferred from its spectroscopic properties.

O-Benzylation of **22** by using *tert*-BuOK or NaOH with phase-transfer catalysis gave quantitatively **14c** and **14a**, respectively. Aminolysis of **22** afforded **24** in 95% yield. Reduction of the latter with $(CH_3)_3N{\cdot}BH_3$ gave the crystalline hydrochloride of **25** in 81% yield. Analysis of the reaction mixture revealed the presence of only small amounts of the 2,3-dihydroindole analogues of **24** and **25**. Apparently, efficient conversion of **24** into **25** is possible, in contrast to the attempted reductions of **15** and **16**. This was not entirely unexpected; we had noted earlier[3a,18] that free oximes are more readily reduced than the corresponding O-benzylated derivatives, a difference we ascribe to steric hindrance.

Formation of the Dioxopiperazines. Acylation of **19** or **25** with pyruvoyl chloride[29] gave **26** and **27**,[30] respectively (Scheme V). These compounds were not isolated but converted in situ into the dioxopiperazines **28** and **29**, respectively, by CF_3COOH treatment.[6b] Surprisingly, **27** gave in addition to **29** (50% yield) a byproduct (26% yield) to which structure **31** was assigned on the basis of spectroscopic evidence; **29** is an intermediate in the conversion of **27** into **31** because prolonged treatment of **29** with CF_3COOH or HCl gave **31** in quantitative yield.

Scheme V



The *O*-benzyl derivative **26** showed analogous behavior: treatment with a trace of CF_3COOH gave **28** whereas treatment with an excess of CF_3COOH gave complete conversion into **30** (76% yield). The latter compound was converted into **31** by catalytic hydrogenation using Pd/C. Definite proof of structures **30** and **31** was obtained by an X-ray crystallographic analysis of **30** (vide infra).

The formation of **30** and **31** can be rationalized as involving the generation of a carbonium ion by protonation of the exomethylene bond, followed by intramolecular alkylation of the indole ring.[31] This reaction might involve a C(3) spiroindolenine derivative as an intermediate.[32]

Finally, compound **29** was easily converted into the neoechinulin analogue **32**; treatment with *p*-toluenesulfonyl

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(30) From a model reaction, i.e., acetylation of **25**, we learned that selective N-acylation occurs if pyridine is used as base. Acylation of free *N*-hydroxyamino acids with stronger bases such as triethylamine is known to give a mixture of N- and O-acylated products: Zinner, E.; Krüger, U. *Chem.-Ztg.* **1976**, *12*, 546. Shin, C.; Nanjo, K.; Ando, E.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 3109. Kolase, T.; Chimiak, A. *Tetrahedron* **1974**, *30*, 3591.

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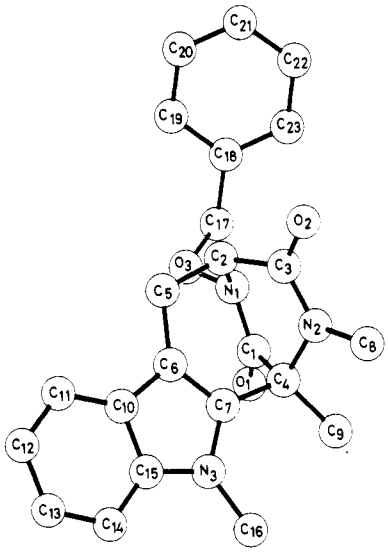

Figure 2. Molecular structure of **30**.

chloride and a slight excess of base gave **32** in 67% yield.

X-ray Crystallographic Analysis of 30. So that the assignment of structure **30** based upon spectroscopic evidence could be confirmed, a single-crystal X-ray structure determination was performed.[33-38] This compound crystallizes from ethyl acetate as triclinic, colorless crystals. A stereoscopic view of the molecules in the unit cell and the molecular structure are shown in Figures 1 and 2, respectively. No significant deviations from expected bond distances and angles were observed. The dihedral angles C4-C1-N2/C1-N1-C3-N2 and N1-C2-C3/C1-N1-C3-N2 are 32.9° and 40.6°, respectively. Thus, the dioxopiperazine ring has a markedly folded conformation, due to the three-carbon bridge. An alternative way to express the "fold" of the dioxopiperazine ring is supplied by the magnitude of the dihedral angle C4-C1-N1-C2/C2-C3-N2-C4, which is 134.3°. This value is very similar to that of the disulfide-bridged dioxopiperazine ring in gliotoxin[39] (129° and 132°) and a gliotoxin analogue[40] (134°).

Discussion

The synthesis of **32** demonstrates the utility of the *N*-hydroxytryptophan derivatives **19** and **25** in the synthesis of tryptophan-containing natural products. This provides impetus to repeat the sequence of reactions **20** → **25** → **32**, commencing with a 2-isopentenyl-substituted indole in order to complete the synthesis of neoechinulin B (**7a**). For the synthesis of the sporidesmin skeleton by the reaction sequence **26** → **28** → **11**, conditions are being sought which avoid the unwanted cyclization into **30**. If *N*-methyl-5-chloro-6,7-dimethoxyindole[41] can be converted into the corresponding dehydrodioxopiperazine intermediate (see **28**, Scheme V), a novel sporidesmin B (**8a**) synthesis should become feasible. An alternative approach to the dihydropyrroloindoline system system **8** might involve the cyclization of **32**; however, we have as yet not been able to accomplish this ring closure.

Experimental Section

Melting points were taken on a Köfler hot stage (Leitz-Wetzler) and are uncorrected. Infrared spectra were measured with a Perkin-Elmer spectrophotometer, Model 397. Proton magnetic resonance spectra were measured on a Varian Associates Model T-60 or a Bruker WH-90 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as the solvent. Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer. Thin-layer chromatography (TLC) was carried out by using Merck precoated silica gel F-254 plates (thickness 0.25 mm). Spots with visualized with a UV hand lamp, iodine vapor, ninhydrin, or Cl_2-TDM.[42] Solvent systems used: (A) CCl_4/CH_2Cl_2, 1/9 v/v; (B) $MeOH/CH_2Cl_2$, 8/92 v/v; (C) $MeOH/CH_2Cl_2$, 1/99 v/v; (D) $MeOH/CH_2Cl_2$, 2/98 v/v; (E) $MeOH/CH_2Cl_2$, 4/96 v/v; (F) $MeOH/CH_2Cl_2$, 6/94 v/v; (G) $MeOH/CH_2Cl_2$, 5/95 v/v; (H) $MeOH/CH_2Cl_2$, 1/9 v/v. For column chromatography Merck silica gel H (type 60) was used under slightly increased pressure (10 cmHg). The Miniprep LC (Jobin Yvon) was used for preparative HPLC.

Ethyl α-(Benzyloximino)-β-(indol-3-yl)propanoate (13). *O*-Benzylhydroxylamine hydrochloride (2.36 g, 15 mmol) and *p*-toluenesulfonic acid (100 mg) were added to a solution of indole-3-pyruvic acid (**12**; Aldrich Co.; 3.0 g, 15 mmol) in dry ethanol (150 mL). Subsequently, the water-ethanol azeotrope was slowly distilled at atmospheric pressure by using a vacuum-jacketed Vigreux column (20 cm), during which the reaction was monitored by TLC (eluent CH_2Cl_2). After completion of the reaction, the mixture was allowed to cool to room temperature and concentrated in vacuo. The oily residue was partitioned between CH_2Cl_2 and water, and the organic layer was washed with 1.0 N HCl, 1.0 N NaOH, water, and brine, dried over Na_2SO_4, and concentrated in vacuo to dryness. Column chromatography of the residue (solvent system A) afforded **13** as an oil in 66% yield, which was homogeneous by TLC (eluent CH_2Cl_2; R_f 0.6): exact mass calcd for $C_{20}H_{20}N_2O_3$ *m/e* 336.1474, found 336.1458; ^1H NMR δ 8.1 (br s, 1 H, NH), 7.7-7.0 (m, 4 H, indole C(4)-C(7) H), 7.25 (s, 5 H, C_6H_5), 6.9 (d, 1 H, indole C(2) H), 5.2 (s, 2 H, $CH_2C_6H_5$), 4.1 (q, 2 H, CH_2CH_3), 4.05 (s, 2 H, indole C(3) CH_2); 1.1 (t, 3 H, CH_2CH_3).

α-(Benzyloximino)-β-(*N*-methylindol-3-yl)propanoic Acid (14a). From 13. A solution of **13** (336 mg, 1 mmol) and CH_3I (284 mg, 2 mmol) in Me_2SO (1 mL) was added dropwise to a stirred suspension of pulverized KOH (552 mg, 4 mmol) in Me_2SO (1 mL). The suspension was stirred at room temperature for 30

(33) A crystal of approximate dimensions 0.1 × 0.5 × 0.5 mm was used. It belongs to the triclinic space group $P\bar{1}$ with a = 11.976 (2) Å, b = 11.945 (4) Å, and Z = 2. Unit cell and space group data were obtained from diffractometer measurements. Accurate unit cell dimensions were determined by least-squares methods from 25 general reflections, measured with Mo Kα radiation (graphite crystal monochromator, λ = 0.71069 Å). Mo Kα radiation was used throughout the experimental X-ray work. Additional crystal data are given.[34] The intensities of 9152 reflections, the full sphere with sin $\theta/\lambda < 0.65$, were measured on a CAD4 diffractometer by using an ω-2θ scan and a scan range of 0.75 + 0.35 tan θ°. Intensity and orientation control reflections were measured every 30 min and used to correct the data for primary beam intensity fluctuations and crystal decay. Symmetry-equivalent reflections were averaged, which resulted in a reflection set consisting of 4576 independent reflections [2753 reflections with $I > 2\sigma(I)$ (counting statistics) and 1823 reflections with $I < 2\sigma(I)$]. These data were corrected for Lorentz and polarization effects and reduced to $|F_o|$ values. The structure was solved by direct methods (MULTAN), using the PDP-8/CSP programs,[35] and refined by full-matrix least-squares methods. In the final cycles, the hydrogen atoms, which could be located in a difference synthesis, were included at calculated positions with a fixed thermal parameter but not refined. Anisotropic thermal parameters were used for all nonhydrogen atoms. The quantity minimized was $\sum(|F_o| - K|F_c|)^2$. The final R value is 0.042 for 332 variables and 2753 contributing reflections. A final difference map showed only densities less than 0.2 e Å^3 and no significant features. The atomic scattering factors for O, N, and C were those of Cromer and Mann[36] and for H were those of Stewart et al.[37] The CSP and XRAY[38] programs were used for the crystallographic calculations. The final atomic parameters are given.[34]

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min under argon. The mixture was quenched by quick addition of water (10 mL), and then the pH was adjusted to 3 by addition of 1 N HCl. The mixture was then extracted with three portions of CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to give **14a** as an oil: 95% yield; ^1H NMR δ 8.6 (br s, 1 H, CO_2H), 7.25 (s, 5 H, C_6H_5), 7.6–6.8 (m, 4 H, indole C(4)–C(7) H,), 6.75 (s, 1 H, indole C(2) H), 5.25 (s, 2 H, $CH_2C_6H_5$), 4.05 (s, 2 H, indole C(3) CH_2), 3.55 (s, 3 H, NCH_3). This compound was further characterized as its methyl ester **14b**.

From 22: A solution of **22** (260 mg, 1 mmol) and benzyl bromide (172 mg, 1 mmol) in ethanol-free CH_2Cl_2 (5 mL) was added to an aqueous solution of NaOH (10 mL of a 50% solution) and a catalytic amount of tetrabutylammonium chloride. The resulting two-phase system was vigorously stirred at room temperature for 1 h. Then CH_2Cl_2 was added, the organic layer was washed with 1 N HCl and with brine, and dried over Na_2SO_4. Evaporation of the solvent gave quantitatively **14a**, which was homogeneous by TLC.

Methyl α-(Benzyloximino)-β-(*N*-methylindol-3-yl)-propanoate (14b). A solution of diazomethane in diethyl ether (20 mL of a 0.3 M solution, 6 mmol) was added dropwise to an ice-cooled, stirred solution of **14a** (511 mg, 1.6 mmol) in methanol (25 mL). The mixture was stirred for 10 min at 0 °C, after which the excess of diazomethane was removed by a stream of argon. Concentration in vacuo gave quantitatively **14b** (538 mg, 1.6 mmol), which was homogenous by TLC (R_f 0.40; solvent system A): ^1H NMR δ 7.25 (s, 5 H, C_6H_5), 7.7–6.8 (m, 4 H, indole C-(4)–C(7) H), 6.8 (s, 1 H, indole C(2) H), 5.35 (s, 2 H, $CH_2C_6H_5$), 4.05 (s, 2 H, indole C(3) CH_2), 3.70 (s, 3 H, OCH_3), 3.60 (s, 3 H, NCH_3).

Ethyl α-(Benzyloximino)-β-(*N*-methylindol-3-yl)-propanoate (14c). From 13. Methyl iodide (4.20 mg, 3 mmol), Ag_2O (700 mg, 3 mmol), and K_2CO_3 (414 mg, 3 mmol) were added to a stirred solution of **13** (250 mg, 0.75 mmol) in DMF (10 mL) at room temperature under argon. The mixture was stirred at room temperature for 2 days under argon. It was then filtered through Celite and concentrated to dryness in vacuo (5 mm Hg, bath temperature 40 °C). The oily residue was partitioned between CH_2Cl_2 and water, and the organic layer was washed with 1 N HCl and brine and dried over Na_2SO_4. Concentration in vacuo afforded a yellow oil, which was subjected to column chromatography (Merck silica gel, solvent system A) to yield **14c** (65%). The product thus obtained was homogeneous by TLC (R_f 0.40; solvent system A): ^1H NMR δ 7.25 (s, 5 H, C_6H_5), 7.6–6.7 (m, 4 H, indole C(4)–C(7) H), 6.7 (s, 1 H, indole C(2) H), 5.25 (s, 2 H, $CH_2C_6H_5$), 4.2 (q, 2 H, OCH_2CH_3), 4.0 (s, 2 H, indole C(3) CH_2), 3.65 (s, 3 H, NCH_3), 1.2 (t, 3 H, OCH_2CH_3).

From 22. Benzyl bromide (1.3 g, 7.6 mmol) was added dropwise to a stirred solution of **22** (1.8 g, 6.9 mmol) in dimethoxyethane (40 mL) at room temperature under argon. Stirring under argon was continued at room temperature for 3 h. The precipitated sodium bromide was then removed by filtration through Celite and the filtrate concentrated to dryness in vacuo. The residue was partitioned between CH_2Cl_2 and water, and the organic layer was washed with 1 N HCl and with brine and dried over Na_2SO_4. Evaporation of the solvent in vacuo gave quanitatively **14c**, which was homogeneous by TLC.

α-(Benzyloximino)-β-(indol-3-yl)-*N*-methylpropanamide (15). An aqueous solution of $MeNH_2$ (10 mL of a 35% solution) was added all at once to a stirred solution of **13** (336 mg, 1 mmol) in dioxane (10 mL) at room temperature under argon. The mixture was stirred at room temperature for 16 h under argon. The solvents were then removed in vacuo. The residue was dissolved in CH_2Cl_2; this solution was washed with 1 N HCl, dried over Na_2SO_4, and concentrated to dryness to yield **15** (93%) which was homogeneous by TLC (R_f 0.10; CH_2Cl_2): ^1H NMR δ 8.3 (m, 1 H, indole NH), 7.3 (s, 5 H, C_6H_5), 7.8–6.9 (m, 4 H, indole C(4)–C(7) H), 6.9 (d, 1 H, indole C(2) H), 6.6 (m, 1 H, amide NH), 2.75 (d, 3 H, NCH_3).

α-(Benzyloximino)-β-(*N*-methylindol-3-yl)-*N*-methyl-propanamide (16). The esters **14b** (538 mg, 1.58 mmol) and **14c** (405 mg, 1.20 mmol) were aminolyzed as described for the preparations of **15** from **13** to afford **16** in yields of 86% and 90%, respectively. The compounds thus obtained were identical and homogeneous on TLC (R_f 0.31 solvent system B): ^1H NMR δ 7.3 (s, 5 H, C_6H_5), 7.8–6.9 (m, 4 H, indole C(4) C(7) H, 6.9 (s, 1 H, indole C(2) H), 6.6 (m, 1 H, NH), 5.2 (s, 2 H, $CH_2C_6H_5$), 3.6 (s, 3 H, indole *N*-CH_3), 2.75 (d, 3 H, $NHCH_3$).

Ethyl α-(Benzyloxamino)-β-(indol-3-yl)propanoate (17). A solution of HCl in ethanol (5 mL of a 7 N solution) was added to a stirred solution of **13** (200 mg, 0.6 mmol) and triethylamine borohydride complex (Aldrich Chemical Co.; 50 mg, 0.7 mmol) in ethanol (5 mL) at room temperature. Stirring was continued for 24 h at room temperature. The mixture was then concentrated to dryness in vacuo and the residue partitioned between CH_2Cl_2 and water. The organic layer was washed with 1 N NaOH and with brine, dried over Na_2SO_4, and then concentrated in vacuo. The residue was subjected to column chromatography (Merck silica gel, solvent system C) to yield **17** (50%), which was homogeneous on TLC (R_f 0.26; solvent system C): exact mass calcd for $C_{20}H_{22}N_2O_3$ *m/e* 338.1630, found 338.1608; ^1H NMR δ 8.0 (m, 1 H, indole NH), 7.6–7.0 (m, 4 H, indole C(4)–C(7) H), 7.3 (s, 5 H, C_6H_5), 6.96 (d, J = 2.6 Hz, 1 H, indole C(2) H), 4.72 (s, 2 H, $CH_2C_6H_5$), 4.13 (q, 2 H, OCH_2CH_3), 3.97 (X part of ABX spectrum, 1 H, J_{AX} = 7.3 Hz, J_{BX} = 6.5 Hz, indole C(3) CH_2CH), 3.06 (3 lines, 2 H, AB part of ABX spectrum, indole C(3) CH_2CH), 1.15 (t, 3 H, OCH_2CH_3).

Ethyl α-(Benzyloxamino)-β-(*N*-methylindol-3-yl)-propanoate (18). The oxime **14c** (1.05 g, 3 mmol) was reduced with triethylamine borohydride (1.2 g, 3.6 mmol) as described for the synthesis of **17** from **13**. Column chromatography (Merck silica gel, CH_2Cl_2 as eluent) gave **18** in 43% yield (0.452 g, 1.3 mmol), which was homogeneous by TLC (R_f 0.60; solvent system D): ^1H NMR δ 7.2 (s, 5 H, C_6H_5), 7.6–6.9 (m, 4 H, indole C(4)–C(7) H), 6.75 (s, 1 H, indole C(2) H), 5.9 (m, 1 H, NH), 4.65 (s, 2 H, $CH_2C_6H_5$), 4.1 (q, 2 H, OCH_2CH_3), 3.9 (X part of ABX spectrum, 1 H, indole C(3) CH_2CH), 3.6 (s, 3 H, NCH_3), 3.05 (AB part of ABX spectrum, 2 H, indole C(3) CH_2CH), 1.1 (t, 3 H, OCH_2CH_3).

α-(Benzyloxamino)-β-(*N*-methylindol-3-yl)-*N*-methyl-propanamide (19). The ester **18** (401 mg, 1.14 mmol) was treated with methylamine as described for the preparation of **15** from **13** to yield **19** (54%) after column chromatography (Merck silica gel, solvent system C). The compound thus obtained was homogeneous by TLC (R_f 0.42; solvent system E): ^1H NMR δ 7.25 (s, 5 H, C_6H_5), 7.7–6.9 (m, 4 H, indole C(4)–C(7) H), 6.75 (s, 1 H, indole C(2) H), 6.4 (m, 1 H, $NHCH_3$), 5.7 (m, 1 H, NH–O), 4.55 (s, 2 H, $CH_2C_6H_5$), 3.9–3.4 (m, 1 H, X part of ABX spectrum, indole C(3) CH_2–CH), 3.6 (s, 3 H, indole NCH_3), 3.2–2.8 (m, 2 H, AB part of ABX spectrum, indole C(3) CH_2), 2.6 (d, 3 H, $NHCH_3$).

Ethyl α-(Hydroxyimino)-β-bromopropanoate (21a). The procedure used for the synthesis of this compound is similar to that reported earlier[18] and is more efficient than the one employed by Gilchrist et al.[28] Hydroxylamine hydrochloride (3.48 g, 50 mmol) was added to a stirred solution of ethyl bromopyruvate (Aldrich Chemical Co.; 9.75 g, 50 mmol) in $CHCl_3$ (150 mL) and CH_3OH (100 mL) at room temperature. The mixture was then stirred at room temperature for 16 h and concentrated to dryness. The residue was dissolved in CH_2Cl_2, washed with 0.1 N HCl and with brine, and dried over Na_2SO_4. Evaporation of the solvent in vacuo gave quantitatively crystalline **21a**, which was recrystallized from CH_2Cl_2–*n*-hexane; mp 78–79 °C (lit.[28] mp 76–78 °C). The compound was homogeneous on TLC (R_f 0.42; solvent system F): ^1H NMR δ 10.0 (br s, 1 H, NOH), 4.30 (q, 2 H, OCH_2CH_3), 4.25 (s, 2 H, CH_2Br), 1.3 (t, 3 H, OCH_2CH_3).

Ethyl α-(Benzyloximino)-β-bromopropanoate (21b). Ethyl bromopyruvate (6.0 g, 25 mmol) was treated with *O*-benzylhydroxylamine hydrochloride (4.0 g, 25 mmol) as described for the preparation of **21a** to yield **21b** quantitatively. The compound was homogeneous on TLC (R_f 0.77; CH_2Cl_2): ^1H NMR δ 7.2 (s, 5 H, C_6H_5), 5.25 (s, 2 H, $CH_2C_6H_5$), 4.2 (q, 2 H, OCH_2CH_3), 4.05 (s, 2 H, CH_2Br), 1.2 (t, 3 H, OCH_2CH_3).

Ethyl α-(Hydroxyimino)-β-(*N*-methylindol-3-yl)-propanoate (22). A solution of **21a** (7.35 g, 35 mmol) in CH_2Cl_2 (75 mL) was added dropwise to a stirred solution of **20** (15 g, 105 mmol) and a suspension of Na_2CO_3 (7.5 g, 70 mmol) in CH_2Cl_2 (75 mL) at room temperature under argon. Stirring was continued at room temperature for 16 h under argon. The mixture was then filtered through Celite and concentrated to dryness. The residue was subjected to column chromatography (Merck silica gel, CH_2Cl_2) to yield crystalline **22** (88%), which was recrystallized

from CH_2Cl_2/*n*-hexane; mp 118–120 °C. The compound was homogeneous by TLC (R_f 0.40; solvent system F): ^1H NMR δ 10.2 (br s, 1 H, NOH), 7.7–6.9 (m, 4 H, indole C(4)–C(7) H), 6.8 (s, 1 H, indole C(2) H), 4.2 (q, 2 H, OCH_2CH_3), 4.05 (s, 2 H, indole C(3) CH_2), 3.6 (s, 3 H, NCH_3), 1.3 (t, 3 H, OCH_2CH_3).

Treatment of **22** (520 mg, 2 mmol) with another 1 equiv of **21a** (420 mg, 2 mmol) and Na_2CO_3 (530 mg, 5 mmol) as described above gave quantitatively the 2:1 adduct **23**, which was homogeneous by TLC (R_f 0.30; solvent system F): electron-impact mass spectrum, exact mass calcd for $C_{14}H_{16}N_2O_3$ *m/e* 260.1161, found 260.1189; ^1H NMR 9.8 (br s, 1 H, NOH), 7.3–6.3 (m, 4 H, indoline C(4)–C(7) H), 5.5 (s, 1 H, indolin C(2) H), 4.4–3.9 (2 q, 4 H, 2 OCH_2CH_3), 3.3 and 2.5 (4 lines, 2 H, AB spectrum, J_{AB} = 16 Hz, indoline C(3) C(α)H_2), 3.2 (s, 2 H, indoline C(3) C(β)H_2), 3.0 (s, 3 H, NCH_3), 1.4–1.1 (2 t, 6 H, 2 OCH_2CH_3).

α-(Hydroxyimino)-β-(*N*-methylindol-3-yl)-*N*-methylpropanamide (24). The ester **22** (401 mg, 1.14 mmol) was aminolyzed as described for the preparation of **15** from **13** to give crystalline **24** in 95% yield, which was homogeneous by TLC (R_f 0.27; solvent system F). This amide could be recrystallized from CH_2Cl_2: mp 163–167 °C; ^1H NMR δ 10.4 (br s, 1 H, NOH), 7.8–6.8 (m, 4 H, indole C(4)–C(7) H), 6.7 (s, 1 H, indole C(2) H), 6.6 (m, 1 H, NH), 4.05 (s, 2 H, indole C(3) CH_2), 3.45 (s, 3 H, indole NCH_3), 2.6 (d, 3 H, $NHCH_3$); electron-impact mass spectrum, exact mass calcd for $C_{13}H_{15}N_3O_2$ *m/e* 245.1164, found 245.1187.

α-(Hydroxyamino)-β-(*N*-methylindol-3-yl)-*N*-methylpropanamide (25). A solution of HCl in ethanol (20 mL of a 7 N solution) was added to a stirred solution of **24** (4.56 g, 18.6 mmol) and triethylamine–borohydride (Aldrich Chemical Co.; 1.46 g, 20.0 mmol) in ethanol (20 mL) at room temperature. Stirring was continued for 24 h at room temperature. The mixture was then concentrated to dryness in vacuo and the residue triturated with CH_2Cl_2 to give the amorphous hydrochloride of **25**. Subsequently, the free hydroxyamine was obtained by adding aqueous $NaHCO_3$ (3 mL of a 5% solution) to a suspension of the salt in CH_2Cl_2. The organic layer was washed with brine and dried with Na_2SO_4. Evaporation of the solvent in vacuo and column chromatography of the residue (Merck silica gel, solvent system G) gave **25** in 81% yield, which was homogeneous by TLC (R_f 0.36; solvent system H): mp 126–128 °C (CH_2Cl_2/*n*-hexane); electron-impact mass spectrum, exact mass calcd for $C_{13}H_{17}N_3O_2$ *m/e* 247.1321, found 247.1413; ^1H NMR δ 7.68–7.0 (m, 4 H, indole C(4)–C(7) H), 6.93 (s, 1 H, indole C(2) H), 6.65 (m, 1 H, NH–CH), 4.3 (m, 2 H, NHOH), 3.84–3.68 (m, 1 H, HON(H)CH), 3.73 (s, 3 H, indole NCH_3), 3.39–2.71 (8 lines, AB part of ABX spectrum, J_{AB} = 15 Hz, J_{AX} = 5 Hz, indole C(3) CH_2), 2.8 (d, 3 H, $NHCH_3$).

1-(Benzyloxy)-2,5-dioxo-3-methylene-4-methyl-6-[(*N*-methylindol-3-yl)methyl]piperazine (28) and 8-(Benzyloxy)-7,9-dioxo-5,6-dimethyl-6,8-diazabicyclo[3.2.2]nonano[4,5-*b*]-*N*-methylindole (30). Pyruvoyl chloride[29] (2.0 mmol, 220 mg) and subsequently triethylamine (2.0 mmol, 200 mg) were added to a stirred and chilled (0 °C) solution of **19** (1.96 mmol, 660 mg) in CH_2Cl_2 (dry and free of alcohol, 20 mL) under argon. Stirring was continued for 20 h at room temperature. The reaction mixture was washed with 0.1 N HCl(aq), and, subsequently, 1 drop of CF_3COOH was added at room temperature. The resulting solution was stirred at room temperature for 2 h, washed with 1 N NaOH and with brine, dried over Na_2SO_4, and concentrated to dryness. The resulting material was used for the preparation of **30** without further purification: TLC R_f 0.50 (solvent system F); ^1H NMR δ 7.4 (s, 5 H, C_6H_5), 7.4–7.0 (m, 4 H, indole C(4)–C(7) H); 6.8 (s, 1 H, indole C(2) H), 5.05 and 4.95 (AB spectrum, 2 H, $OCH_2C_6H_5$), 3.9 and 5.1 (2 d, 2 H, C=CH_2), 4.4 (X part of ABX spectrum, 1 H, NC(H)C(O)), 3.65 (s, 3 H, indole NCH_3), 3.4 (AB part of ABX spectrum, 2 H, indole C(3) CH_2CH), 2.5 (s, 3 H, N(4) CH_3). The material thus obtained was dissolved in $CHCl_3$ (25 mL) and CF_3COOH (1 mL). The reaction mixture was stirred at room temperature for 3 h, washed with 1 N NaOH and with brine, and dried over Na_2SO_4. The residue, obtained by evaporation of the solvent in vacuo, was chromatographed (Merck silica gel column, solvent system C) to yield **30** (582 mg, 76%), which was recrystallized from ethyl acetate: mp 157–159 °C; TLC R_f 0.43 (solvent system D); electron-impact mass spectrum, exact mass calcd for $C_{23}H_{23}N_3O_3$ *m/e* 389.1739, found 389.1747; UV (ethanol) λ_{max} 227.290 nm; IR ($CHCl_3$) ν_{max} 1685 cm^{-1}; IR (KBr) ν_{max} 1700, 1680 cm^{-1}; ^1H NMR δ 7.39 (s, 4 H, C_6H_5), 7.49–6.99 (m, 4 H, indole C(4)–C(7) H), 5.09 and 4.98 (AB spectrum, 2 H, J_{AB} = 10.5 Hz, $OCH_2C_6H_5$), 4.47 (X part of ABX spectrum, 1 H, NC(H)C(O)), 3.90 (s, 3 H, indole NCH_3), 3.38 and 3.16 (AB part of ABX spectrum, 2 H, J_{AB} = 17.3 Hz, J_{AX} = 3.8 Hz, J_{BX} = 2.6 Hz, indole C(3) CH_2–CH), 2.94 (s, 3 H, N(9) CH_3), 2.24 (s, 3 H, C(3) CH_3). Anal. $C_{23}H_{23}N_3O_3$: C, H, N.

1-Hydroxy-2,5-dioxo-3-methylene-4-methyl-6-[(*N*-methylindol-3-yl)methyl]piperazine (29) and 8-Hydroxy-7,9-dioxo-5,6-dimethyl-6,8-diazabicyclo[3.2.2]nonano[4,5-*b*]-*N*-methylindole (31). Pyruvoyl chloride[29] (10.5 mmol, 1.115 g) and subsequently pyridine (10.0 mmol, 790 mg) were added to a stirred and chilled (0 °C) solution of **25** (10 mmol, 2.47 g) in CH_2Cl_2 (dry and free of alcohol, 100 mL) under argon. The reaction mixture was stirred at room temperature under argon for 4 h; the formation of **27** was monitored by TLC (solvent system H, $FeCl_3$ positive). The volume was reduced to about half under reduced pressure, and then 1 mL of CF_3CO_2H was added at room temperature. The resulting clear solution was stirred at room temperature for 16 h, washed with two portions of brine (10 mL), dried over Na_2SO_4, and concentrated to dryness. Column chromatography (Merck silica gel, solvent system G) of the residue yielded a mixture of **29** and **31** (75%) in a 2:1 ratio (based on ^1H NMR spectroscopy). Careful rechromatography gave **29** (866 mg, 29%), which was homogeneous by TLC (solvent system H). The remaining fractions were combined and concentrated in vacuo to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and divided in two equal portions. Into one portion was bubbled dry HCl, and into the other one was added CF_3COOH (1 mL) at room temperature. Both reactions were monitored by TLC (solvent system H) and were recombined upon completion of the reaction. Washing with two portions of brine, drying over Na_2SO_4, and evaporation of the solvent gave quantitatively (46% from **25**) compound **31**. Compounds **29** and **31** gave on TLC the characteristic red spots for hydroxamic acids upon spraying with $FeCl_3$(aq).

Compound 31 from 30. To a solution of **30** (102 mg, 0.26 mmol) in CH_3OH (25 mL) Pd/C (10 mg) was added. The resulting suspension was treated with H_2 at 2 bar and at room temperature in a Parr apparatus. The reaction was complete after 2 h. Filtration and evaporation of the solvent gave quantitatively **31** (78 mg).

Compound 29: TLC R_f 0.62 (solvent system H); mp 157–162 °C (CH_2Cl_2); electron-impact mass spectrum, *m/e* 299 (M^+ for $C_{16}H_{17}N_3O_3$); UV (ethanol) λ_{max} 220, 284 nm; IR ($CHCl_3$) ν_{max} indole C(4)–C(7) H), 6.8 (s, 1 H, indole C(2) H), 5.1 and 4.0 (2 d, 2 × 1 H, C=CH_2); 4.7 (X part of ABX spectrum, 1 H, NC(H)C(O)), 3.6 (s, 3 H, indole NCH_3), 3.55 (AB part of ABX spectrum, indole C(3) CH_2CH), 2.5 (s, 3 H, N(4) CH_3).

Compound 31: TLC R_f 0.60 (solvent system H); mp 220–226 °C dec (CH_2Cl_2); electron-impact mass spectrum, exact mass calcd for $C_{16}H_{17}N_3O_3$ *m/e* 299.1270, found 299.1263; UV (ethanol) λ_{max} 221.288 nm; IR ($CHCl_3$) ν_{max} 1680 cm^{-1}; IR (KBr) ν_{max} 1695, 1655, 1640 cm^{-1}; ^1H NMR δ 9.5 (br s, 1 H, NOH), 7.48–7.0 (m, 4 H, indole C(4)–C(7) H), 4.74 (X part of ABX spectrum, 1 H, NC(H)C(O)), 3.67 (s, 3 H, indole NCH), 3.58 and 3.18 (AB part of ABX spectrum, 2 H, J_{AB} = 18.0 Hz, J_{AX} = 4 Hz, J_{BX} = 3 Hz, indole C(3) CH_2CH), 2.83 (s, 3 H, N(9) CH_3), 2.04 (br s, 3 H, C(3) CH_3). Anal. $C_{16}H_{17}N_3O_3$: C, H, N.

2,5-Dioxo-3-methylene-4-methyl-6-[(*N*-methylindol-3-yl)-methyl]piperazine (32). *p*-Toluenesulfonyl chloride (536 mg, 2.82 mmol) and pyridene (265 mg, 2.90 mmol) were added to a stirred and chilled (0 °C) solution of **29** (800 mg, 2.68 mmol) in DMF (20 mL) under argon. The reaction mixture was stirred at room temperature for 16 h under argon, after which the solvent was evaporated in vacuo. The residue was partitioned between CH_2Cl_2 and water, and the organic layer was dried over Na_2SO_4. Evaporation of the solvent and column chromatography of the residue (Merck silica gel, solvent system G) gave **32** in 67% yield (499 mg). Recrystallization from CH_2Cl_2–*n*-hexane gave light yellow crystals: mp 248–252 °C dec; TLC R_f 0.63 (solvent system H); electron-impact mass spectrum, *m/e* 281 (M^+ for $C_{16}H_{15}N_3O_2$); UV (ethanol) λ_{max} 222, 278, 378 nm; IR (KBr) 3210 (NH), 1680 (CO), 1615, 1600 (C=C) cm^{-1}; ^1H NMR (CD_2Cl_2) δ 8.0 (br s, 1 H, NH), 7.8–7.1 (m, 4 H, indole C(4)–C(7) H), 7.3 (s, 1 H, C=C—C(H)=C); 7.2 (s, 1 H, indole C(2) H), 5.75 and 4.95 (2 d, 2 × 1 H, C=CH_2), 3.86 (s, 3 H, indole NCH_3), 3.32 (s, 3 H, N(4)

CH_3). Anal. $C_{16}H_{15}N_3O_2$: C, H, N.

Acknowledgment. We thank Dr. T. F. Spande and Professor Dr. R. J. F. Nivard for reading and criticizing the manuscript.

Registry No. 12, 392-12-1; **13**, 81095-79-6; **14a**, 81095-80-9; **14b**, 81095-81-0; **14c**, 81095-82-1; **15**, 81095-83-2; **16**, 81095-84-3; **17**, 81095-85-4; **18**, 81095-86-5; **19**, 81095-87-6; **20**, 603-76-9; **21a**, 73472-94-3; **21b**, 81095-88-7; **22**, 81095-89-8; **23**, 81120-61-8; **24**, 81095-90-1; **25**, 81095-91-2; **25**·HCl, 81095-92-3; **26**, 81095-93-4; **27**, 81095-94-5; **28**, 81120-62-9; **29**, 81095-95-6; **30**, 81095-96-7; **31**, 81120-63-0; **32**, 81095-97-8.

Supplementary Material Available: Tables I and II of crystal data, final positional parameters, and thermal parameters (2 pages). Ordering information is given on any current masthead page.

Structure of Humistratin: A Novel Cardenolide from the Sandhill Milkweed *Asclepias humistrata*[1]

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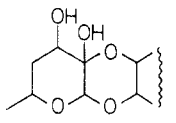
Received October 30, 1981

Humistratin (**5**), a new Δ^7-cardenolide monoglycoside of an ecological interest, was isolated from the leaves and stems of the sandhill milkweed *Asclepias humistrata* Walt. (Asclepiadaceae). Its structure and stereochemistry were elucidated by IR, EIMS, CIMS, ^1H NMR, ^{13}C NMR, and X-ray crystallography. Its hypothetical sugar, 4,6-dideoxy-β-D-*glycero*-D-*glycero*-2-hexosulopyranose, is doubly linked at 1 and 2, through acetal (glycosidic) and hemiketal bonds, to positions 3β and 2α, respectively, of its hypothetical genin, 2α,3β,14-trihydroxy-19-oxo-5α,14β-carda-7,20(22)-dienolide, to form a dioxane ring with the resultant chiral center at C(2) of the sugar moiety *S*.

Cardenolides constitute one of several groups of plant secondary compounds that are sequestered by phytophagous insects for defense against predation.[2] Most members of the milkweed genus *Asclepias* (Asclepiadaceae) produce these cardioactive steroids at varying concentrations.[3] In the southeastern region of the United States, the sandhill milkweed *Asclepias humistrata* is one of the most common milkweeds,[4] serving as an abundant food source for several insect herbivores,[5] including larvae of the Monarch butterfly *Danaus plexippus*.[6] When larvae are reared on leaves of this milkweed, the resultant butterflies contain high levels of cardenolides (ca. 0.4% dry weight expressed as digitoxin equivalents)[7] and are highly emetic to the blue jay *Cyanocitta cristata bromia*.[8] However, no investigation has been undertaken on the chemical structure of any individual cardenolide stored in those butterflies or originally present in their food plant. Preliminary TLC analysis[5] has shown that humistratin, the most concentrated cardenolide in the leaves of *A. humistrata*, is the one stored at the highest level in *A. humistrata* fed Monarch butterflies. We report here the isolation and structural elucidation of humistratin.

The scheme of isolation was based on that employed by Reichstein et al.[9] The hot aqueous methanol extract of leaves and stems of *A. humistrata* was partitioned between water and organic solvents of increasing polarity. The chloroform partition layer, into which the major portion of humistratin had been transferred from the aqueous suspension, was further fractionated by column chromatography to give needle crystals of humistratin in a yield of 0.018%.

Humistratin gave a strong positive osazone reaction for methylreductinic acid, and its electron-impact mass spectrum had two prominent peaks at m/z 128 and 113, assignable to methylreductinic acid ($C_6H_8O_3$) and its demethylation product, respectively. These facts are considered[10,11] to constitute strong evidence that the cardenolide glycoside may contain a 4,6-dideoxyhexosulose moiety which is doubly conjugated to the aglycon, thereby producing a dioxane ring, as depicted in A. Such a doubly



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linked 4,6-dideoxyhexosulose, first formulated by Coombe and Watson[12] for the sugar moiety in gomphoside, has also

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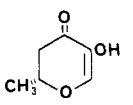

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