a homogenous solution. The solution was kept at room temperature for 3 days, then it was diluted with water and extracted with ethyl acetate. The product was prepurified on TLC and gave 76 mg of solids. Purification on a Celite partition column (isooctane–methanol, 1:9) gave 21 mg of (20S)-20-hydroxycholesterol (**4a**), mp 130–132 °C (MeOH), ir and NMR identical with those of an authentic sample (Table I). In addition there was obtained 43 mg of 17α-hydroxycholesterol, mp 175–177 °C (MeOH); the ir and NMR spectra were indistinguishable from those of an authentic sample[14] (Table I).

(20S)-3β,17α,20-Trihydroxycholest-5-ene (17a) from 3d. To a solution of 200 mg of the 3,5-cyclo derivative (**3d**) was added 250 mg of osmium tetroxide and the mixture was kept for 5 days in the dark. Then it was poured into a solution of 500 mg of lithium aluminum hydride in 140 ml of ether and the mixture was heated under reflux for 3 h. The excess hydride was decomposed with a saturated aqueous solution of sodium sulfate and the crude product isolated in the usual fashion. The total residue was hydrolyzed with perchloric acid in the same fashion as indicated (above) for the alcohol **4a**. The product, crystallized from methylene chloride, gave 25.0 mg with mp 160–162 °C;[15] the ir and NMR spectra were superimposable on those obtained from authentic material (Table I).

Acknowledgment. This investigation was supported by Grants AM-12172 and AM-03419 from the National Institutes of Health and GB-38612 from the National Science Foundation. The authors are grateful to Mrs. D. N. Davis for some of the NMR spectra, and some of the microanalyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N.Y.

Registry No.—**2a**, 21903-19-5; **2b**, 56312-72-2; **3a**, 41083-88-9; **3d**, 59873-54-0; **4a**, 516-72-3; **4b**, 7484-20-0; **5a**, 7484-22-2; **5b**, 7429-99-4; **8a**, 59905-87-2; **8b**, 54548-85-5; **10b**, 3092-00-0; **11b**, 5143-83-9; **11c**, 58449-04-0; **12b**, 59873-55-1; **12c**, 59873-56-2; **16d**, 2867-93-8; **17a**, 382-78-5; dihydropyran, 25512-65-6; 1-bromo-3-methylbutane, 107-82-4; (Z)-3β-tetrahydropyranyloxy-22(R)-methoxycholesta-5,17(20)-diene, 59873-57-3; (Z)-3β-tetrahydropyranyloxy-22(S)-methoxycholesta-5,17(20)-diene, 59873-58-4; (Z)-3α,5-cyclo-6β-acetoxy-5α-cholest-17(20)-ene, 59873-59-5.

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Approaches to Analogues of Dehydrogliotoxin. 6.[1] An Efficient Synthesis of a Gliotoxin Analogue with Anti-Reverse Transcriptase Activity[2]

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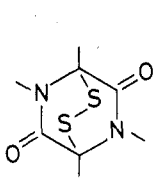
The addition of α-ketoacyl chlorides **4** to indolenine-2-carboxamides **3**, followed by spontaneous, diastereoselective ring closure to 3,6-disubstituted dioxopiperazines (**5** → **6**), provides an efficient, new synthesis of gliotoxin analogues. Compound **6** was converted into the mercaptoalkene **11** by treatment with H_2S. Regiospecific and diastereoselective addition of H_2S to the exo methylene group gave cis dithiol **12**. This zinc ion catalyzed reaction is believed to proceed via the chelate intermediate **19a**. Several oxidation procedures were studied for the conversion of **12** into disulfide **13**. The tri- and tetrasulfides **21** and **22** were obtained from **12** by reaction with SCl_2 and S_2Cl_2, respectively; the monosulfide **20** was obtained from **13** by treatment with $(C_6H_5)_3P$. Analogies between this synthesis and what is known about the biosynthesis of gliotoxin are discussed. Compound **13** thus obtained (81% overall yield) was found to inhibit the enzyme reverse transcriptase, while having no effect on transcriptase; its activity is comparable to that of gliotoxin.

The epidithiodioxopiperazine system **1**, common to a number of fungal metabolites, including dehydrogliotoxin (**2**), the sporidesmins, aranotins,[5] and others,[6] appears to be the site of the potent antiviral, antibacterial, or antifungal activities of this group of compounds. Several syntheses of simple derivatives of **1** have appeared[7] and Kishi and coworkers have recently reported a 12-step synthesis of (±)-dehydrogliotoxin (**2**).[8] We wish to report the development of



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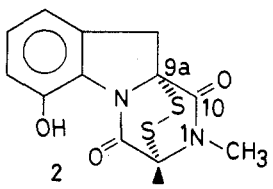

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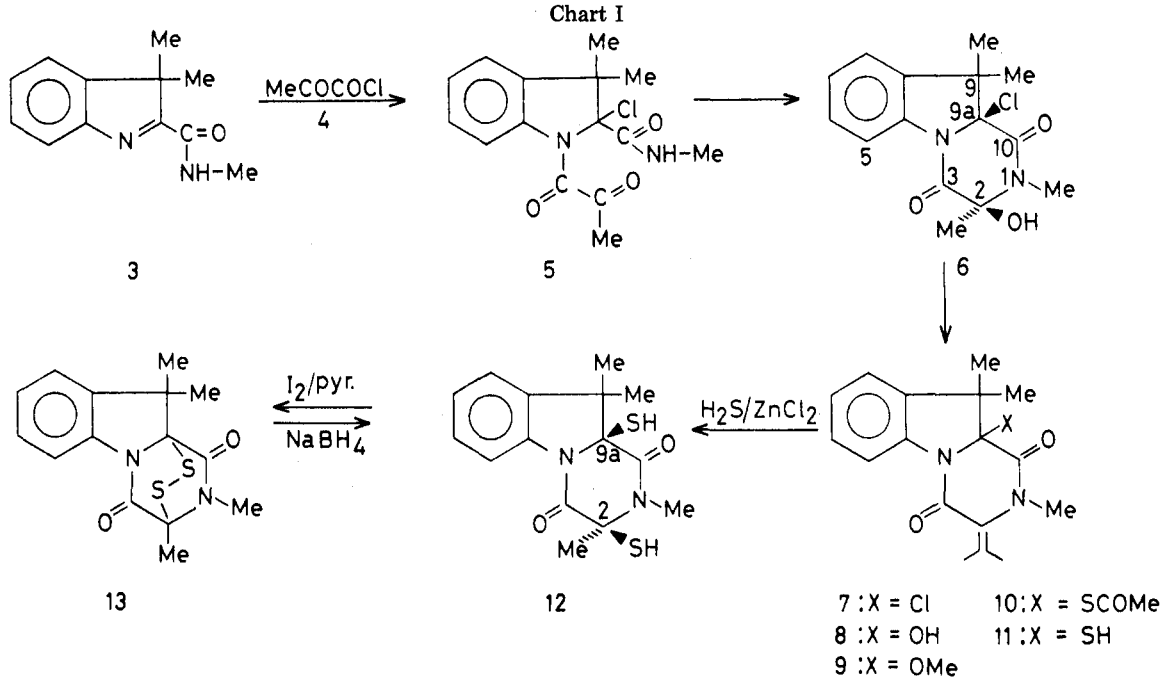
Chart I



a scheme which forms the epipolythiodioxopiperazines **13** and **20–22** in a high yield, three-step, one-pot reaction under mild conditions, and which may be of general applicability for other dehydrogliotoxin analogues.

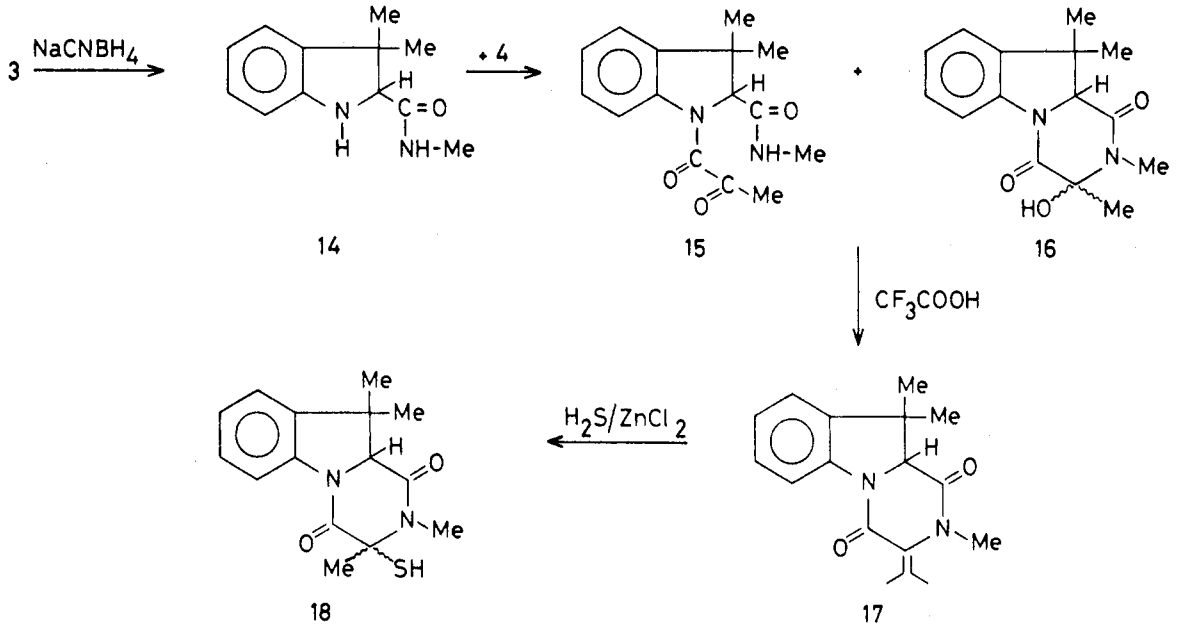
Although dehydrogliotoxin (**2**) can be viewed as an oxidized condensation product of a 2-mercaptoindoline-2-carboxylic acid and an α-mercapto-α-amino acid derivative, neither of these components is evidently capable of independent existence. We have evidence[9] that unacylated indoline-2-thiols are inherently unstable and, so far as we know, no unacylated α-mercapto-α-amino acid has yet been synthesized. Accordingly, we felt that a synthetic procedure for this system would have to create a functional group at the indoline C_2 position, convertible to a mercapto group, simultaneously with the acylation of the indoline nitrogen by an α-mercapto-α-amino acid equivalent. Our initial synthetic approach involved[9] the ring closure ($N_1 \rightarrow C_{10}$) of a *seco*-gliotoxin analogue, prepared in this manner. This reaction failed, apparently because the necessarily strenuous reaction conditions we employed were incompatible with a strained ring system. For this reason we turned to a scheme featuring a preformed dioxopiperazine ring but having groups at the α positions capable of being converted to mercapto groups. Two particular reactions, run consecutively, could, we thought, create such an intermediate (Chart I): (1) the addition (**3** → **5**) of acyl chlorides (e.g., **4**) to the imine bond of indolenines **3** and (2) the intramolecular cyclization (**5** → **6**) of an amide nitrogen with the α-carbonyl group of an α-ketoacyl residue. The former is a general reaction discovered by Leuchs,[10] later employed[11] by Wieland in the synthesis of an *N*-acylindoline 2-thioether and recently by us[9] as a convenient route to 1-acyl-2-mercaptoindoline-2-carboxylic acid esters. Reaction 2, discovered by Bergmann and Grafe,[12] who studied intermolecular reactions of primary or secondary amides with pyruvates, has been employed by us[1] as a practical route to α-mercapto-α-acylamidocarboxylic acids.

After we had developed a practical preparation of the hitherto elusive pyruvoyl chloride (**4**),[13] these two synthetic ideas were applied as follows. Pyruvoyl chloride (**4**) and the indolenine carboxamide **3** in CCl_4 reacted within 50 min at room temperature to form the Leuchs' adduct **5**. About 5 h after mixing, this intermediate was converted completely into **6** and appeared to be only one stereoisomer by ^1H NMR spectroscopy.

This diastereoselective[14] ring closure of **5**, which has been observed recently by others[15,16] in closely related systems, has been rationalized by Häusler and Schmidt[15] and is predicted to give the cis product **6**. After our work had been completed,[2] however, a report by Poisel and Schmidt[17] suggested that the reaction might not be stereoselective in all cases.

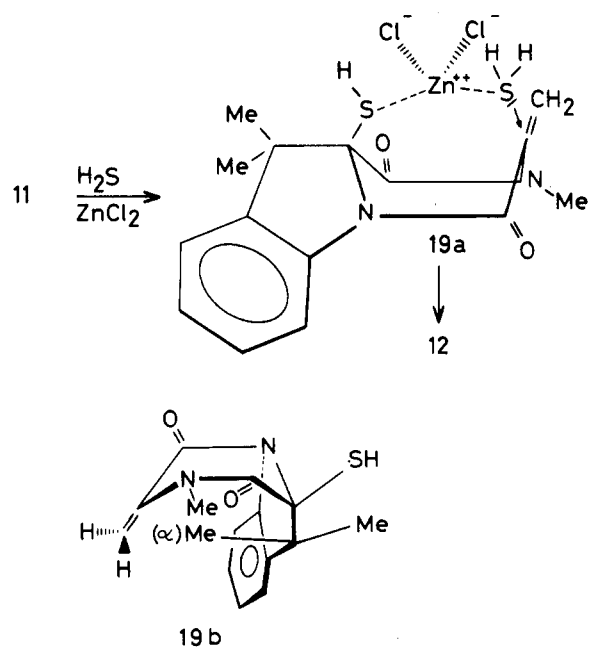
We first attempted the direct conversion of **6** to the dithiol **12** by treatment with H_2S. However, **6** was found to be unstable; when stirred for 10 h it is transformed into a mixture of **7** and **8**, the latter apparently arising from the water produced on spontaneous dehydration. The chloroalkene **7** could be converted quantitatively into **8** with 1 equiv of H_2O in pyridine, while treatment with MeOH/CCl_4 or thioacetic acid and BF_3·Et_2O in CH_2Cl_2 gave **9** (96%) and **10** (45%), respectively. When H_2S was bubbled through a CH_2Cl_2 solution of **6** or **7** for 1 h at room temperature, the mercaptoalkene **11** resulted. We then faced the problem of converting **11** into **12**. Recently, Machin and Sammes[18] and Marshall et al.[16b] showed that only in the presence of strong acids are sulfur nucleophiles added in α fashion across the double bond of dehydro cyclodipeptides, whereas under weakly acidic conditions β-addition is observed. When **11** is exposed to H_2S in the presence of boron trifluoride etherate or *p*-TosOH, an intractable reaction mixture resulted. When, however, the reaction of **11** is conducted in an all-glass pressure flask containing liquid H_2S at room temperature with CF_3COOH as catalyst, a 50% yield of the cis dithiol **12** resulted besides unidentifiable material. Although this avoids the time-consuming process of bubbling H_2S through a CH_2Cl_2 solution of **11** (where reaction also occurs), we felt that the general applicability of our scheme would be compromised by these acidic reaction conditions and consequently we searched for milder ones. We reasoned that H_2S might be converted into a strong enough acid to catalyze this reaction by chelation with transition metal ions; zinc chloride was chosen for the initial study[19] and was tested with the model compound **17**, which was prepared in the following manner (Chart II). Reduction of **3** with $NaCNBH_3$, followed by reaction with **4** in the presence of the Hünig base, gave a 3:1 mixture of **15** and **16**, respectively; treatment with a trace of CF_3COOH gave **17** in 83% overall yield. When alkene **17** was allowed to react with liquid H_2S in the presence of $ZnCl_2$, only one α-substituted stereoisomer **18** (unknown stereochemistry) resulted quantitatively.

Chart II



Encouraged by this result, we exposed the mercaptoalkene **11** to these conditions, and it yielded quantitatively the α adduct **12**. This reaction was also found to proceed in a diastereoselective fashion, as only the cis dithiol could be detected. The possibility that a trace of HCl, from hydrolysis of $ZnCl_2$, was the true catalyst could be excluded by the observation that dry HCl only gave an intractable reaction mixture. The zinc chloride catalyzed regiospecific and diastereoselective addition reaction could be explained in the following way. A zinc complex with the C_{9a} SH group in **11**[19] might direct the incoming SH groups from the same face by complexation (**19a**, Chart III), yielding **12**. Cis addition could also be explained if **19b** were to represent the preferred conformation of **11**. This conformer relieves the interaction of NCH_3 with an exomethylene hydrogen in **19a** but shields the α-face of the exomethylene group by the 9(α)-methyl group. Although slightly nonplanar (5–10°) amide bonds in dioxopiperazines have precedent,[20a] the significant deviation from planarity implied in **19b** may make it a less likely explanation for the observed stereochemistry.

Chart III

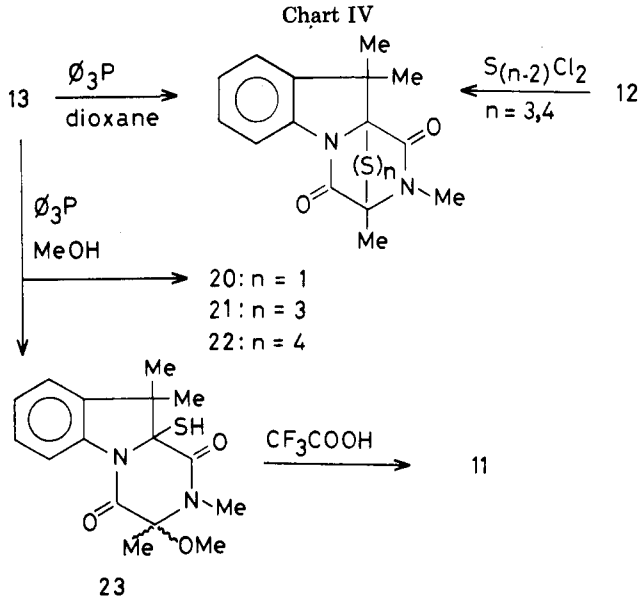


We do have some evidence that the cis configuration of **12** may be thermodynamically more stable than the trans form, as the above-mentioned CF_3COOH-catalyzed reaction of **11** with H_2S gave *cis*-**12** (50%) besides unidentifiable products among which no trans isomer of **12** could be detected.[20b] We have no firm evidence, however, that equilibration occurs under these conditions.

The cis orientation of the C_2 and C_{9a} thiol groups in **12** is proved by its ready oxidation to the epidithiodioxopiperazine **13**. This oxidation can be performed by simply bubbling air through an aqueous methanol solution of **12** in the presence of traces of $ZnCl_2$[21] (37% overall yield from **3**). The yield could be improved slightly (51%) by using KI_3 as an oxidant in a two-phase system.[7b,22] Finally the method of choice for this oxidation was discovered to be I_2 in CH_2Cl_2 in the presence of pyridine under anhydrous conditions. This raised the overall yield of **13** to 81% after column chromatography and made the three-step route (**3** → **7** + **8** → **12** → **13**) a truly practical, one-pot synthesis.

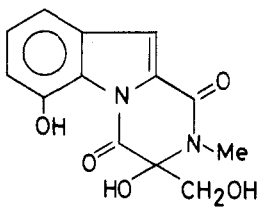
The disulfide **13** could be reduced to the dithiol **12** (80%) by treatment with $NaBH_4$ in C_2H_5OH.[5] Reaction with $(C_6H_5)_3P$[23] in dioxane gave the strained monosulfide **20** in 93% yield (Chart IV); using methanol instead of dioxane in this

Chart IV

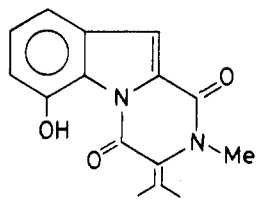


reaction yielded besides **20** (33%) the ring-opened **23** (63%), which could be converted into **11** by treatment with CF_3COOH. The formation of **23**[24] indicates a regioselective attack of the phosphine on the less hindered sulfur atom of **13**. The dithiol **12** could be converted into the trisulfide **21** or tetrasulfide **22** by treatment with SCl_2[25a] or S_2Cl_2,[25b] respectively. At room temperature **21** exists in two conformations, as was concluded from the ^1H NMR spectrum. A similar observation has been reported on the trisulfide sporidesmin E.[26]

The similarity of structures **6** and **7** to the metabolites **24** and **25**, which are postulated to be intermediates in the bio-



24



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synthesis of gliotoxin,[5] is apparent. This, together with the facts that the route **3** → **13** involves highly stereoselective, high-yield reactions and can be carried out at room temperature and neutral pH, tempts us to speculate that our sequence could be a biomimetic one.

Biological Activity. Compound **13** was found to inhibit reverse transcriptase, the RNA-dependent DNA polymerase of RNA tumor viruses. Thus, in the presence of 3.9×10^{-4} M (130 μg/ml) and 3.9×10^{-5} M (13 μg/ml) of **13**, the poly A-dependent incorporation of 3H-dTMP residues in an enzyme preparation derived from Rauscher leukemia virus was 14 and 41% of the blank activity, respectively.[27] This activity is of the same order of magnitude as that for gliotoxin. The latter inhibited endogenous reverse transcriptase activity of Rauscher sarcoma virus: with 50 μg/ml, 25% of the enzyme activity remained.[28]

Earlier we found that an analogue of **13**, having a methylene sulfide bridge, was devoid of antiviral and antibacterial activity.[29] These results again lend support to the proposal that natural products containing the epidithiodioxopiperazine moiety require the disulfide bridge for biological activity.

No activity of **13** on the transcriptase (DNA-dependent RNA polymerase) of *E. coli* bacteria was found.[30] This selectivity is of interest as another epidithiodioxopiperazine, i.e. acetylaranotin, is a highly selective inhibitor of transcriptase.[24b]

Experimental Section

Infrared spectra were measured with a Perkin-Elmer spectrophotometer, Model 257. Proton magnetic resonance spectra were measured on a Varian Associates Model A-100 spectrometer. Chemical shifts are reported as δ values (ppm) relative to hexamethyldisiloxane as an external standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing Varian Associates SMI-B spectrometer (electron impact), or a Finnigan mass spectrometer 1015D with Model 6000 data system (chemical ionization). Melting points were taken on a Köfler hot state (Leitz-Wetzlar) and are uncorrected. Thin layer chromatography (TLC) was carried out using Merck precoated silica gel 60F-254 plates, thickness 0.25 mm. Spots were visualized with a uv hand lamp, iodine vapor and, in the case of sulfur-containing products, by spraying with 2% aqueous $AgNO_3$.[24b]

***N*-Methyl-3,3-dimethylindolenine-2-carboxamide (3).** A solution of 2.17 g (10 mmol) of ethyl 3,3-dimethylindolenine-2-carboxylate[9] in 30 ml of dimethoxyethane containing methylamine (8 M) was kept at 80 °C in an autoclave for 16 h (pressure 11–12 atm). Evaporation of the solvent and excess reagent gave a crystalline mass which was recrystallized from hexane, to give **3** in 90% yield: mp 109–110 °C; ir ($CHCl_3$) 3410 (NH), 1670 (amide), and 1545 cm^{-1} (C=N); NMR (CCl_4) δ 7.87 (m, 1 H, C_7H), 7.65 (m, 3 H, C_{4-6} H), 3.28 (d, 3 H, NCH_3) and 1.80 (s, 6 H, 2 C_3CH_3). Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.97; N, 13.86. Found: C, 71.3; H, 7.0; N, 13.9.

9,9a-Dihydro-1,9,9-trimethyl-2-methylene-3,10-diketo-9a-chloropiperazino[1,2-*a*]indole (7) and 9a-Hydroxy Analogue 8. To a stirred solution of 1.01 g (5 mmol) of **3** in 25 ml of dry CCl_4 was added at room temperature 586 mg (5.5 mmol) of pyruvoyl chloride (**4**).[13] After stirring for 5 h at room temperature the ring closure product **6** had formed quantitatively, as was shown by infrared and ^1H NMR spectroscopy: ir (CCl_4) 3600–3100 (OH) and 1685 cm^{-1} (br), C=N band had disappeared; NMR (CCl_4) δ 8.32 (m, 1 H, C_5 H), 7.63 (m, 3 H, C_{6-8}H), 6.44 (s, br, 1 H, OH), 3.49 (s, 3 H, NMe), 2.18 (s, 6 H, C_9 $C_\alpha H_3$ and C_2 CH_3) and 1.60 (s, 3 H, C_9 $C_\beta H_3$).

After stirring for 10 h, **6** was converted completely into a mixture of **7** and **8** (varying ratios) which is poorly soluble in CCl_4. The addition of 4Å molecular sieves to a solution of **6** did not prevent the formation of **8**.

Chloroalkene **7**: ir ($CHCl_3$) 1695 cm^{-1} (amide); NMR δ 8.65 (m, 1 H, C_5 H), 7.75 (m, 3 H, C_{6-8} H), 6.54 (d, 1 H, C=CH_α), 5.66 (d, 1 H, C=CH_β), 3.76 (s, 3 H, NMe), 2.27 (s, 3 H, C_9 $C_\alpha H_3$), and 1.72 (s, 3 H, C_9 $C_\beta H_3$).

Hydroxyalkene **8**: mp 178–183 C; ir ($CHCl_3$) 3600–3100 (OH) and 1690 cm^{-1} (amide); NMR δ 8.65 (m, 1 H, C_5 H), 7.75 (m, 3 H, C_{6-8} H), 6.44 (d, 1 H, C=CH_α), 5.53 (d, 1 H, C=CH_β), 3.68 (s, 3 H, NMe), 2.13 (s, 3 H, C_9 $C_\alpha H_3$) and 1.62 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum *m/e* 272 (M^+), 255 (M^+ − OH), and 240 (M^+ − OH − CH_3).

9,9a-Dihydro-1,9,9-trimethyl-2-methylene-3,10-diketo-9a-methoxypiperazino[1,2-*a*]indole (9). Excess MeOH (20 ml) was added to a stirred solution of 291 mg (1 mmol) of **7** in 5 ml of CCl_4 at oom temperature. After stirring for 1 h, solvents and excess reagent were removed in vacuo, to yield 280 mg (96%) of oily residue: NMR δ 8.24 (m, 1 H, C_5 H), 7.40 (m, 3 H, C_{6-8} H), 6.25 (d, 1 H, C=CH_α), 5.37 (d, 1 H, C=CH_β), 3.55 (s, 3 H, OMe), 3.35 (s, 3 H, NMe), 1.93 (s, 3 H, C_9 $C_\alpha H_3$) and 1.39 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum *m/e* 286 (M^+), 271 (M^+ − CH_3), 255 (M^+ − OCH_3), and 240 (M^+ − CH_3 − OCH_3).

9,9a-Dihydro-1,9,9-trimethyl-2-methylene-3,10-diketo-9a-thioacetylpiperazino[1,2-*a*]indole (10). Thioacetic acid (182 mg, 2.4 mmol) and then 1 drop of BF_3·$(C_2H_5)_2O$ were added to a stirred solution of 582 mg (2 mmol) of **7** in 10 ml of dry CH_2Cl_2 at room temperature. After stirring for 3 h at room temperature, the solvent and excess reagent were removed in vacuo, to yield a crystalline mass, which was recrystallized from MeOH–hexane: mp 167–169 °C; yield 297 mg (45%); TLC (4% C_2H_5OH–toluene), only one spot; ir ($CHCl_3$) 1690 cm^{-1} (br, C=O); NMR δ 8.37 (m, 1 H, C_5 H), 7.57 (m, 3 H, C_{6-8} H), 6.38 (d, 1 H, C=CH_α), 5.45 (d, 1 H, C=CH_β), 3.68 (s, 3 H, NMe), 2.53 (s, 3 H, $SCOCH_3$), 2.09 (s, 3 H, C_9 $C_\alpha H_3$), and 1.51 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum *m/e* 330 (M^+), 287 (M^+ − $COCH_3$), 273 (M^+ − MeN=C=O), and 255 (M^+ − $SCOCH_3$). Anal. Calcd for $C_{17}H_{18}N_2SO_3$: C, 61.80; H, 5.49; N, 8.48. Found: C, 62.0; H, 5.6; N, 8.5.

9,9a-Dihydro-1,9,9-trimethyl-2-methylene-3,10-diketo-9a-mercaptopiperazino[1,2-*a*]indole (11). H_2S saturated with CH_2Cl_2 was bubbled for 2 h through an ice-cooled, stirred CH_2Cl_2 solution of a mixture of **7** and **8** (1 mmol) to which was added a few crystals of anhydrous zinc chloride. The reaction mixture was filtered and the solvent evaporated in vacuo, to yield a crystalline mass, which on TLC (5% CH_3OH–$CHCl_3$) showed only one spot: ir ($CHCl_3$) 2570 (SH) and 1690 cm^{-1} (br, CO); NMR δ 8.56 (m, 1 H, C_5 H), 7.68 (m, 3 H, C_{6-8} H), 6.42 (d, 1 H, C=CH_α), 5.52 (d, 1 H, C=CH_β), 3.71 (s, 3 H, NMe), 3.10 (s, 1 H, SH), 2.18 (s, 3 H, C_9 $C_\alpha H_3$), and 1.68 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum *m/e* 288 (M^+) and 255 (M^+ − SH).

9,9a-Dihydro-1,2,9,9-tetramethyl-2,9a-dimercapto-3,10-diketopiperazino[1,2-*a*]indole (12). From 7 + 8. Dried H_2S (about 10 ml) was condensed at −70 °C into a dry CH_2Cl_2 solution (75 ml) of a mixture of **7** and **8** (5 mmol), to which was added an excess (7 mmol) of anhydrous zinc chloride. The all-glass pressure flask was closed, and the reaction mixture was stirred at room temperature for 16 h, during which time the pressure increased to about 8 atm. Then the flask was opened, the reaction mixture filtered, and the solvent evaporated in vacuo, to yield a glassy material which showed on TLC (2% CH_3OH–CH_2Cl_2) besides a spot on the origin (**12**) a faint spot corresponding to **13** which indicates the easy oxidation of **12**. Formation of **13** was not observed when traces of CH_2Cl_2-soluble zinc ions were removed from the product before bringing on TLC plates: ir ($CHCl_3$) 2570 and 2540 (SH), 1685 cm^{-1} (CO); NMR δ 8.51 (m, 1 H, C_5 H), 7.68 (m, 3 H, C_{6-8} H), 4.27 (s, br, 1 H, SH), 3.60 (s, 3 H, NMe), 3.44 (s, 1 H, SH), 2.42 (s, 3 H, C_2 CH_3), 2.21 (s, 3 H, C_9 $C_\alpha H_3$), and 1.59 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum *m/e* 322 (M^+), 289 (M^+ − SH), 288 (M^+ H_2S), 279 (M^+ − $COCH_3$), 274 (M − SH − CH_3), 273, 261, 260, 256, 255, and 241 (M^+ − SH − SH − CH_3).

From 13. To an ice-cooled stirred solution of 145 mg (0.43 mmol) of **13** in 25 ml of dry C_2H_5OH was added 57 mg (1.5 mmol) of $NaBH_4$ in one portion. After stirring for 10 min at 0 °C another 57-mg portion of $NaBH_4$ was added. Stirring was continued for 20 min at 0 °C and finally 15 min at room temperature. After evaporation of the solvent in vacuo, water and $CHCl_3$ were added and the pH adjusted at 7 with 2 N H_2SO_4. The aqueous layer was extracted twice with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo to give 110 mg (76%) of **12**, identical with the above specimen, except for no tendency toward oxidation on TLC.

9,9a-Dihydro-1,2,9,9-tetramethyl-2,9a-epidithio-3,10-diketopiperazino[1,2-*a*]indole (13). Oxygen Oxidation. Air was bubbled through a solution of 840 mg (2.54 mmol) of **12** (prepared from **7** + **8**) in 15 ml of 80% CH_3OH–H_2O, for 2.5 h at room temperature. Removal of the solvent and chromatography of the residue on Sephadex LH-20 in 80% CH_3OH–H_2O (column 3.4 × 167 cm, flow rate 52 ml/h, 15-ml fractions) afforded 292 mg (36%) of **13** (mp 142–144 °C recrystallized from CH_3OH–H_2O) which was homogeneous by TLC (R_f 0.50, 4% CH_3OH–toluene, R_f 0.57, 2% CH_3OH–CH_2Cl_2): ir ($CHCl_3$) 1692 cm^{-1} (CO); NMR δ 8.37 (m, 1 H, C_5 H), 7.62 (m, 3 H, C_{6-8} H), 3.47 (s, 3 H, NMe), 2.45 (s, 3 H, C_2 CH_3), 2.14 (s, 3 H, C_9 $C_\beta H_3$), and 1.95 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum (electron impact, only peaks with rel intensity >20) *m/e* 320 (M^+, 39), 256 ($M^+ - S_2$, 81), and 241 ($M^+ - S_2 - CH_3$, 100); (chemical ionization, NH_3) *m/e* 338 ($M + NH_4^+$, 33), 321 ($M + H^+$, 100), and 257 ($M + H^+ - S_2$, 71).

Anal. Calcd for $C_{15}H_{16}N_2O_2S_2$: C, 56.23; H, 5.03; N, 8.74; S 20.01. Found: C, 56.0; H, 5.1; N, 8.5; S, 20.1.

Iodine Oxidation. A 2.5% solution of KI_3 in pyridine was added dropwise at room temperature to a solution of 1.61 g (5 mmol) of **12** in 75 ml of dry CH_2Cl_2 until the reaction mixture remained colored. The pyridine salts were removed by filtration, and the filtrate evaporated to dryness. The residue was column chromatographed on 50 g of Merck silica gel PF_{254} in $CHCl_3$–CCl_4 (4:1 v/v) under slightly increased pressure (about 10 cmHg) to afford 1.29 g (4.0 mmol, 81%) of **13** which was identical with the specimen described above.

3,3-Dimethylindoline-2-(*N*-methyl)carboxamide (14). To a solution of 1.01 g (5 mmol) of **3** in 100 ml of absolute ethanol at room temperature was added a trace of bromocresol green; after addition of 2 N methanolic HCl to a yellow end point (pH ≃ 3), the stirred mixture was supplied with 1.5 g (24 mmol) of sodium cyanoborohydride, and more HCl–methanol solution was added to maintain the yellow color. Stirring was continued for 30 min. Then the mixture was concentrated in vacuo, after which chloroform and water were added. The aqueous layer was washed with chloroform and the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to give 1.02 g (100%) of white crystals (mp 143–145 °C) homogeneous on TLC (12% EtOH/toluene): NMR δ 6.93–7.73 (m, 4 H, C_{4-7} H), 4.75 (m, 1 H, NH), 4.40 (d, 1 H, C_2 H), 3.23 (d, 3 H, NMe), 1.94 (s, 3 H, C_3 $C_\alpha H_3$), and 1.46 (s, 3 H, C_3 $C_\beta H_3$).

9,9a-Dihydro-1,9,9-trimethyl-2-methylene-3,10-diketopiperazine[1,2-*a*]indole (17). 4 (4.4 ml, 2.2 mmol) in CCl_4 (0.5 M) was added at room temperature to a stirred solution of 408 mg (2 mmol) of **14** and 285 mg (2.2 mmol) of diisopropylethylamine in 50 ml of dry tetrahydrofuran. After stirring for 16 h at room temperature, 200 ml of $CHCl_3$ was added, after which the reaction mixture was washed with 1 N HCl, 5% $NaHCO_3$, and water until neutral, and then dried (Na_2SO_4). A ^1H NMR spectrum indicated the presence of **15** and **16** in a ratio of 3:1, respectively. After filtration 1 ml of trifluoroacetic acid was added and the solution stirred for 1 h at room temperature. Then solid Na_2CO_3 was added, together with Na_2SO_4. Filtration, then concentration in vacuo, followed by column chromatography on 30 g of Merck silica gel PF_{254} in chloroform gave 425 mg (83%) of **17**, which showed only one spot on TLC (12% C_2H_5OH–toluene): mp 139–141 °C (CCl_4); NMR δ 8.45 (m, 1 H, C_5 H), 7.53 (m, 3 H, C_{6-8} H), 6.20 (d, 1 H, C=CH_α), 5.30 (d, 1 H, C=CH_β), 4.77 (s, 1 H, C_{9a} H), 3.90 (s, 3 H, NMe), 2.05 (s, 3 H, C_9 $C_\alpha H_3$), 1.52 (s, 3 H, C_9 $C_\beta H_3$).

Anal. Calcd for $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.0; H, 6.2; N, 10.6.

9,9a-Dihydro-1,2,9,9-tetramethyl-2-mercapto-3,10-diketopiperazino[1,2-*a*]indole (18). A solution of 256 mg (1 mmol) of **17** in 25 ml of dry CH_2Cl_2 was allowed to react with H_2S in the presence of 200 mg of $ZnCl_2$ as described for the preparation of **12**. After filtration and removal of the solvent in vacuo, 290 mg of a glassy material was obtained, which showed only one, $AgNO_3$ positive, spot on TLC (R_f identical with that of **17**): NMR δ 8.43 (m, 1 H, C_5 H), 7.53 (m, 3 H, C_{6-8} H), 4.75 (S, 1 H, C_{9a} H), 3.70 (s, 3 H, NMe), 3.12 (s, br, 1 H, SH), 2.45 (s, 3 H, C_2 CH_3), 2.06 (s, 3 H, C_9 $C_\alpha H_3$), and 1.52 (s, 3 H, C_9 $C_\alpha H_3$).

9,9a-Dihydro-1,2,9,9-tetramethyl-2,9a-epithio-3,10-diketopiperazino[1,2-*a*]indole (20). A solution of 64 mg (0.2 mmol) of **13** and 57 mg (0.22 mmol) of triphenylphosphine in 15 ml of dry dioxane was stirred for 30 min at room temperature. Evaporation of the solvent in vacuo and column chromatography on 17 g of Merck silica gel PF_{254} in $CHCl_3$–CCl_4 (4:1 v/v) gave 54 mg (93%) of crystalline (mp 99–101 °C, CH_3OH) material which on TLC showed only one spot (R_f 0.58, 2% MeOH/CH_2Cl_2): ir ($CHCl_3$) 1721 cm^{-1} (CO); NMR δ 7.91 (m, 1 H, C_5 H), 7.60 (m, 3 H, C_{6-8} H), 3.32 (s, 3 H, NMe), 2.19 (s, 3 H, C_2 CH_3), 2.13 (s, 3 H, C_9 $C_\alpha H_3$), and 1.85 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum (electron impact) *m/e* 288 (M^+), 273 ($M^+ - CH_3$), and 256 ($M^+ - S$); (chemical ionization, NH_3) 306 ($M + NH_4^+$, 100), 289 ($M + H^+$, 28), 276 ($M + NH_4^+ - 2CH_3$, 31), 274 ($M + NH_4^+ - S$, 7), 259 ($M + H^+ - 2CH_3$, 50), and 257 ($M + H^+ - S$, 14).

Anal. Calcd for $C_{15}H_{16}N_2O_2S$: C, 62.48; H, 5.59; N, 9.71. Found: C, 62.5; H, 5.7; N, 9.7.

9,9a-Dihydro-1,2,9,9-tetramethyl-2-methoxy-3,10-diketo-9a-mercaptopiperazino[1,2-*a*]indole (23). When the above reaction was performed in methanol instead of dioxane, another compound besides **20** (33%) was obtained after silica gel column chromatography. This product (63%) was assigned structure **23** on the basis of the following data: ir ($CHCl_3$) 1680 cm^{-1} (CO); NMR δ 8.40 (m, 1 H, C_5 H), 7.60 (m, 3 H, C_{6-8} H), 3.78 (s, 3 H, OCH_3), 3.56 (s, 1 H, SH), 3.45 (s, 3 H, NMe), 2.18 (s, 6 H, C_2 CH_3 + C_9 $C_\alpha H_3$), and 1.54 (s, 3 H, C_9 $C_\alpha H_3$); mass spectrum *m/e* 320 (M^+), 288, 273, 260, 256 ($M - SH - OCH_3$), 241, and 231.

Treatment of **23** with trifluoroacetic acid in CCl_4 gave quantitatively the mercaptoalkene **11**.

9,9a-Dihydro-1,2,9,9-tetramethyl-2,9a-epitrithio-3,10-diketopiperazino[1,2-*a*]indole (21). A solution of 47 mg (0.43 mmol) of SCl_2 in 3 ml of ethanol-free $CHCl_3$ was added dropwise to an ice-cooled, stirred solution of 140 mg (0.43 mmol) of **12** in 10 ml of ethanol-free $CHCl_3$. After stirring for 30 min at room temperature, the reaction mixture was washed with 5% $NaHCO_3$ solution and water until neutral and then dried (Na_2SO_4). Evaporation, followed by column chromatography on the residue on 20 g of Merck silica gel PF_{254} in $CHCl_3$–CCl_4 (4:1 v/v), yielded 133 mg (81% of crystalline material, mp 135–136 °C (CH_3OH–H_2O). The ^1H NMR spectrum indicated the presence of two isomers. On TLC (R_f 0.61, 2% MeOH/CH_2Cl_2) only one spot was visible: ir ($CHCl_3$) 1682 cm^{-1} (CO); NMR δ 8.51 and 8.76 (2 m, 1 H, C_5 H), 7.65 (m, 3 H, C_{6-8} H), 3.47 and 3.65 (2 s, 3 H, NMe), 2.38 and 2.42 (2 s, 3 H, C_2 CH_3), 2.12 (s, 3 H, C_9 $C_\alpha H_3$), 1.67 and 1.69 (2 s, 3 H, C_9 $C_\beta H_3$); mass spectrum (chemical ionization, NH_3) *m/e* 370 ($M^+ + NH_4^+$, 46), 353 ($M^+ + H^+$, 8), 338 ($M^+ + NH_4^+ - S$, 100), 321 ($M^+ + H^+ - S$, 37), 257 ($M + H^+ - 3S$, 72).

Anal. Calcd for $C_{15}H_{16}N_2O_2S_3$: C, 51.11; H, 4.58; N, 7.95. Found: C, 51.1; H, 4.8; N, 7.5.

9,9a-Dihydro-1,2,9,9-tetramethyl-2,9a-epitetrathio-3,10-diketopiperazino[1,2-*a*]indole (22). Dithiol **12** (140 mg, 0.43 mmol) was treated with S_2Cl_2 (58 mg, 0.43 mmol) in $CHCl_3$ as described for the preparation of **21**. Column chromatography on silica gel gave 122 mg (67%) of **22**, which, though homogeneous on TLC (R_f 0.59, 2% MeOH/CH_2Cl_2), resisted crystallization. An aqueous methanolic solution of **22** was found to be unstable at room temperature, as was shown by TLC: ir ($CHCl_3$) 1670 cm^{-1} (CO); NMR δ 8.55 (m, 1 H, C_5 H), 7.70 (m, 3 H, C_{6-8} H), 3.57 (s, 3 H, NMe), 2.41 (s, 3 H, C_2 CH_3), 21.9 (s, 3 H, C_9 $C_\alpha H_3$), and 1.69 (s, 3 H, C_9 $C_\beta H_3$); mass spectrum (electron impact) *m/e* 256 ($M^+ - S_4$) and 241 ($M^+ - S_4 - CH_3$); (chemical ionization, NH_3) *m/e* 402 ($M + NH_4^+$, 48), 385 ($M + H^+$, 83), 370 ($M + NH_4^+ - S$, 32), 353 ($M + H^+ - S$, 43), 338 ($M + NH_4^+ - S_2$, 22), 321 ($M + H^+ - S_2$, 32), 289 ($M + H^+ - S_3$, 5), and 257 ($M + H^+ - S_4$, 100).

Anal. Calcd for $C_{15}H_{16}N_2O_2S_4$: C, 46.8; H, 4.2; N, 7.3. Found: C, 46.7; H, 4.2; N, 7.0.

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Registry No.—**3,** 58788-11-7; **4,** 5704-66-5; **6,** 59888-42-5; **7,** 59888-43-6; **8,** 59888-44-7; **9,** 59888-45-8; **10,** 59888-46-9; **11,** 59888-47-0; **12,** 59888-48-1; **13,** 59888-49-2; **14,** 59888-50-5; **15,** 59888-51-6; **16,** 59888-52-7; **17,** 59888-53-8; **18,** 59888-54-9; **20,** 59888-55-0; **21,** 59888-56-1; **22,** 59888-57-2; **23,** 59888-58-3; ethyl 3,3-dimethylindolenine-2-carboxylate, 41296-09-7; methylamine, 74-89-5; thioacetic acid, 507-09-5.

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A New Systematic Degradation of Nicotine to Determine Activity at C-2′ and C-5′. The Pattern of Labeling in Nicotine and Nornicotine Formed from [2-^{14}C]Ornithine in *Nicotiana glutinosa*, and in Nicotine Obtained from *N. tabacum* Exposed to [^{14}C,^{13}C]Carbon Dioxide

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Radioactive nicotine has been degraded by the following sequence: nicotine → cotinine → *cis*-5′-phenylnicotine → benzoic acid [C-5′] + nicotinic acid → barium carbonate [C-2′]. The structure of 5′-phenylnicotine was confirmed by an unambiguous synthesis. On applying this degradation to nicotine and nornicotine isolated from *N. glutinosa* plants which had been fed [2-^{14}C]ornithine, equal labeling was found at C-2 and C-5′ of the pyrrolidine ring of both these alkaloids. Nicotine isolated from *N. tabacum* plants which had been exposed to [^{14}C,^{13}C]carbon dioxide also had equal labeling at C-2′ and C-5′. All these results are thus consistent with the formation of the pyrrolidine ring of nicotine and nornicotine from ornithine via a symmetrical intermediate.

It is more than 20 years since Byerrum[2] and I[3] first reported that ornithine (1) is a precursor of the pyrrolidine ring of nicotine (2). By chemical degradations,[4,5] it was established that [2-^{14}C]ornithine yielded nicotine equally labeled at C-2′ and C-5′.[6] These results led to the proposal that the pyrrolidine ring is formed from ornithine via putrescine, *N*-methylputrescine, and an *N*-methyl-Δ^1-pyrrolinium salt.[7] Indeed, enzymes which carry out these metabolic steps have been isolated from tobacco roots.[8] Symmetrical labeling of the pyrrolidine ring is a result of the intermediacy of free putrescine, a symmetrical compound. However, Rapoport and co-workers,[6,9] on the basis of several short-term feeding experiments with $^{14}CO_2$, have suggested that the formation of nicotine from ornithine, via a symmetrical intermediate, may be a minor or aberrant pathway. This proposal was made since, on occasions,[10] the exposure of tobacco plants to $^{14}CO_2$ led to unsymmetrical labeling of the pyrrolidine ring. In particular, unequal labeling was reported at C-2′ and C-5′. On the other hand, Byerrum and co-workers[11] found symmetrical labeling in the pyrrolidine ring of nicotine obtained from *N. glutinosa* and *N. rustica* plants fed $^{14}CO_2$.

It is generally accepted that nicotine is a precursor of nornicotine (3).[12,13] However, the pattern of labeling in nornicotine after feeding [2-^{14}C]ornithine to tobacco has been reported in only one publication,[14] and in this case it was claimed that the pyrrolidine ring of nornicotine was unsymmetrically labeled.[15]

In view of these conflicting results, and possible errors,[16,17] in the methods used for determining the pattern of labeling in the pyrrolidine ring of nicotine, we have now developed a