Approaches to Analogs of Anhydrogliotoxin

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Abstract: The addition of α-halo-α-aminoacyl chlorides to ethyl indolenine-2-carboxylates followed by reaction with sulfur nucleophiles and a final ring closure provides a convenient and new synthetic scheme to analogs of gliotoxin, the simplest of the natural products containing the epidithiodiketopiperazine system. Illustrative of this approach, adducts of ethyl 3,3-dimethylindolenine-2-carboxylate (**17**) with acid chlorides, α-halo acid chlorides, and *N*-trifluoroacetyl-α,α-dichlorosarcosyl chloride (**44**) have been studied. The last adduct when treated with a sulfide–polysulfide mixture gave a monosulfide **49** (30% yield) but no disulfide **50**. Reduction of **49** with $NaCNBH_3$ proceeded stereoselectively to afford mainly the secogliotoxin analog **51** in addition to the diastereoisomer **52**. Cyclization of this mixture presumably led to the strained epimonothiodiketopiperazine **41**, which easily opened to the isomeric lactam **55** in addition to lactam **56** formed by epimerization.

The number of natural products containing the epidithiodiketopiperazine ring **1** continues to grow with the recent reports on the two fungal metabolites chaetocin (**2**)[2] and verticillin A (**3**).[3] Both are highly active against gram-positive bacteria. Chaetocin is cytostatic but lacks antiviral activity, while verticillin A is cytotoxic and active against mycobacteria. Other members of this group of fungal metabolites are the sporidesmins A through G (**4–6**)[4–10] several of which possess potential antibacterial activity, the aranotins (**7–9**)[11–15] and apoaranotins (**10–11**)[16] which have no antibacterial but do have potent antiviral activity,[14] gliotoxin (**12a**),[4,17] an antibiotic, antifungal and antiviral agent, and dehydrogliotoxin (**12b**)[18] with antibacterial activity.

Two other fungal metabolites, chetomin ($C_{31}H_{30}N_6O_6S_4$)[19] and oryzachlorin ($C_{26}H_{31}N_2O_8S_2Cl$),[20] of unknown structure, probably contain the epidithiodiketopiperazine ring. The former is active against gram-positive bacteria[19] and viruses[21] while the latter has only antifungal and antiviral activity.[20]

The mechanism of antiviral action of gliotoxin[22] and aranotin[13] depends upon the specific inhibition of RNA-dependent DNA polymerases from tumor-producing viruses or blocking of the synthesis of viral RNA[21] in the case of chetomin.

Several syntheses of simple epidithiodiketopiperazines have been reported,[21,23–25] which feature the addition of sulfur substituents to a preformed diketopiperazine. Surprisingly, the simple model **1a** is highly active in inhibiting viral RNA synthesis,[21] in support of the view[26] that the activity of the more complex natural products resides in the epidithiodiketopiperazine ring.

Another approach to this ring system started with 2-benzamido-2-mercaptopropanoic acid (**13**) as a possible precursor.[27]

The drastic reaction conditions of all of these methods preclude their successful extension to the polycyclic epidithiodiketopiperazines. A synthetic approach of general applicability, we felt, would feature the initial construction of the disulfide bridge and then ring closure to a bridged diketopiperazine.

The addition of acyl chlorides to indolenines (Chart I), a reaction first reported by Leuchs, who studied compounds **14–16**,[28–30] served as our first step.

The 2-chloro substituent in Leuchs' adducts **18–20** is known to undergo easy nucleophilic displacement, and

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(2) D. Hauser, H. P. Weber, and H. P. Sigg, *Helv. Chim. Acta*, **53**, 1061 (1970).
(3) H. Minato, M. Matsumoto, and T. Katayama, *Chem. Commun.*, 44 (1971).
(4) A. F. Beecham, J. Fridrichsons, and A. M. Mathieson, *Tetrahedron Lett.*, 3131 (1966).
(5) N. Finch, C. W. Gemenden, I. H. C. Hsu, and W. I. Taylor, *J. Amer. Chem. Soc.*, **85**, 1520 (1963).
(6) R. Hodges, J. S. Shannon, and A. Taylor, *J. Chem. Soc. C*, 1803 (1966).
(7) R. Hodges and J. S. Shannon, *Aust. J. Chem.*, **19**, 1059 (1966).
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(10) R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc. C*, 1665 (1969); E. Francis, R. Rahman, S. Safe, and A. Taylor, *ibid.*, 470 (1972).
(11) R. Nagarajan, L. L. Huckstep, D. H. Lively, D. C. DeLong, M. M. Marsh, and N. Neuss, *J. Amer. Chem. Soc.*, **90**, 2980 (1968).
(12) R. Nagarajan, N. Neuss, and M. M. Marsh, *ibid.*, **90**, 6518 (1968).
(13) P. A. Miller, P. W. Trown, W. Fulmor, G. O. Morton, and J. Karliner, *Biochem. Biophys. Res. Commun.*, 33, 220 (1968).
(14) D. B. Cosulich, N. R. Nelson, and J. H. van den Hende, *J. Amer. Chem. Soc.*, **90**, 6519 (1968).
(15) J. W. Moncrief, *ibid.*, **90**, 6517 (1968).
(16) N. Neuss, R. Nagarajan, B. B. Molloy, and L. L. Huckstep, *Tetrahedron Lett.*, 4467 (1968).
(17) M. R. Bell, J. R. Johnson, B. S. Wildi, and R. B. Woodward, *J. Amer. Chem. Soc.*, **80**, 1001 (1958).
(18) G. Lowe, A. Taylor, and L. C. Vining, *J. Chem. Soc. C*, 1799 (1966).
(19) S. Safe and A. Taylor, *J. Chem. Soc. C*, 472 (1972).
(20) A. Kato, T. Salki, S. Suzuki, K. Ando, G. Tamura, and K. Arima, *Jap. J. Antibiot.* **22**, 322 (1969).
(21) P. W. Trown, *Biochem. Biophys. Res. Commun.*, **33**, 402 (1968).
(22) P. A. Miller, K. P. Milstrey, and P. W. Trown, *Science*, **159**, 431 (1968).
(23) H. Poisel and U. Schmidt, *Angew. Chem.*, **83**, 114 (1971); *Chem. Ber.*, **104**, 1714 (1971); **105**, 625 (1972); E. Oehler, H. Poisel, F. Tataruch, and U. Schmidt, *ibid.*, **105**, 635 (1972); E. Oehler, F. Tataruch, and U. Schmidt, *ibid.*, **105**, 3658 (1972).
(24) T. Hino and T. Sato, *Tetrahedron Lett.*, 3127 (1971).
(25) S. G. Svokos and R. B. Angier, German Patent 2,029,306; *Chem. Abstr.*, **74**, 53845 (1971).
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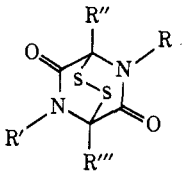
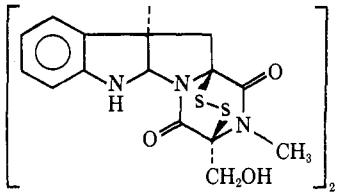
(27) P. M. Pojer and I. D. Rae, *Tetrahedron Lett.*, 3077 (1971); *Aust. J. Chem.*, **25**, 1737 (1972); *cf.* A. Kaneda and R. Sud, *Bull. Chem. Soc. Jap.*, **43**, 2159 (1970).
(28) H. Leuchs, A. Heller, and A. Hoffmann, *Chem. Ber.*, **62**, 871 (1929).
(29) H. Leuchs, G. Wulkow, and H. Gerland, *ibid.*, **65**, 1586 (1932).
(30) H. Leuchs and A. Schlötzer, *ibid.*, **67**, 1572 (1934).



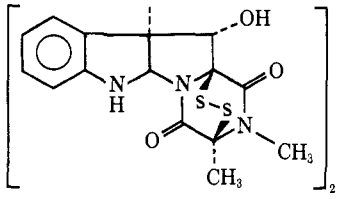
1

a, R = R′ = CH_3; R″ = R‴ = H



2

chaetocin



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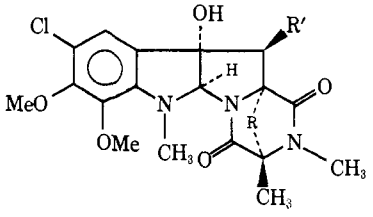
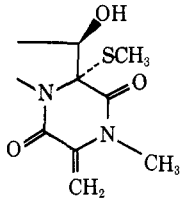
verticillin A



4

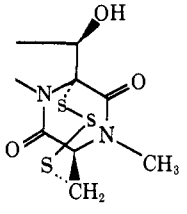
sporidesmin

A, R′ = OH; R = S–S (active)[4]
B, R′ = H; R = S–S (active)[5]
D, R′ = OH; R = SCH_3, CH_3S (inactive)[8]
E, R′ = OH; R = S–S–S (active)[9]
G, R′ = OH; R = S_4[10]



5

sporidesmin F (inactive)[8]



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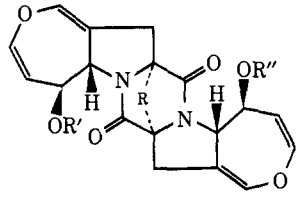
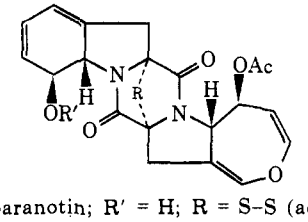
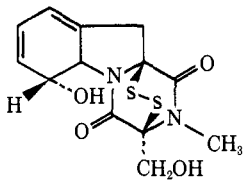
sporidesmin C (active)[7]



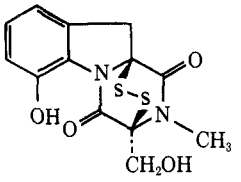
7, aranotin; R′ = H, R″ = Ac; R = S–S (active)
8, acetylaranotin; R′ = R″ = Ac; R = S–S (active)
9, bisdethiodi(methylthio)acetylaranotin; R′ = R″ = Ac; R = CH_3S, SCH_3 (inactive)



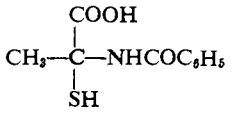
10, apoaranotin; R′ = H; R = S–S (active)
11, bisdethiodi(methylthio)acetylapoaranotin; R′ = Ac; R = –SCH_3, CH_3S– (inactive)



12a, gliotoxin

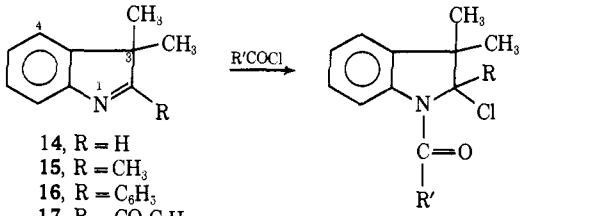


12b, dehydrogliotoxin



13

Chart I

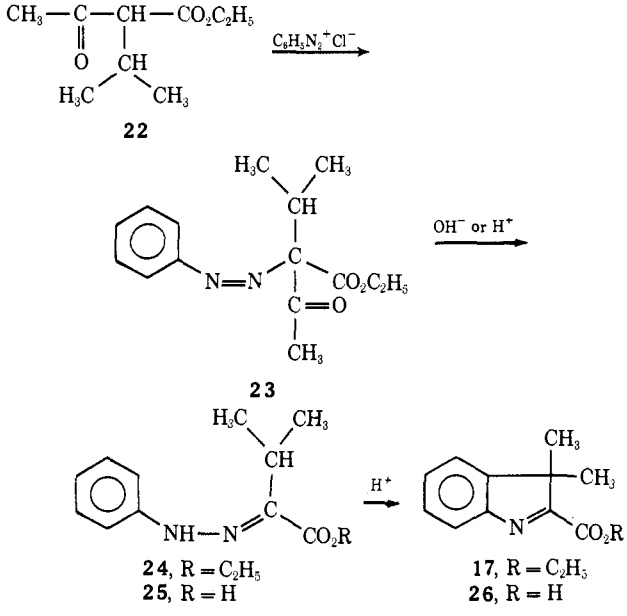


14, R = H
15, R = CH_3
16, R = C_6H_5
17, R = $CO_2C_2H_5$

18, R = H; R′ = CH_3, C_6H_5, or p-$NO_2C_6H_5$
19, R = CH_3; R′ = CH_3 or C_6H_5
20, R = C_6H_5; R′ = C_6H_5
21, R = $CO_2C_2H_5$; R′ = CH_3

reaction of sulfur nucleophiles (*e.g.*, $SCOCH_3^-$, SCN^-, $S_2O_3^{2-}$, etc) on the adduct **21** derived from **17** was first investigated as a route to 1-acylindoline-2-carboxylic acid derivatives having a thio function in the 2 position. The indolenine ester **17** was prepared as outlined in Chart II.

Chart II



22

23

24, R = C_2H_5
25, R = H

17, R = C_2H_5
26, R = H

The azo ester **23**, when prepared from ethyl α-isopropylacetoacetate and benzenediazonium chloride under mildly alkaline conditions, was stable enough to permit isolation. Careful treatment with ethanolic solutions of sodium hydroxide or preferably ammonium hydroxide gave the hydrazone ester **24**, which was converted into **17** by refluxing in HCl-saturated ethanol. The yields are much higher than reported in the published procedure[31] where the coupling and hydrazone

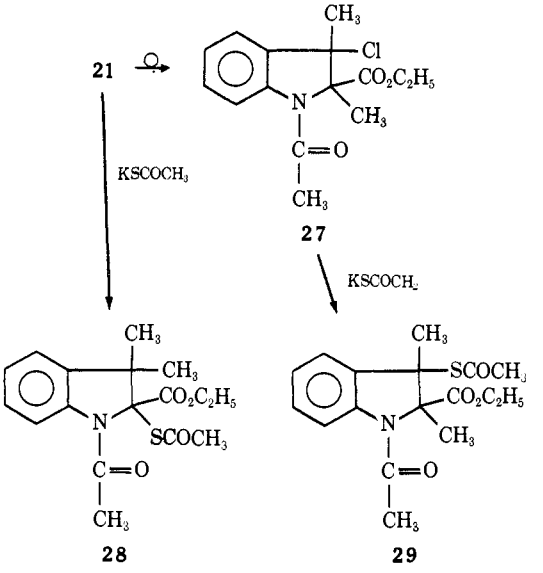
(31) R. Robinson and H. Suginome, *J. Chem. Soc.*, 298 (1932).

formation steps are carried out under such strongly alkaline conditions that only the hydrazone **25** can be isolated. This, on Fischer cyclization, gives a mixture of **17** and **26** accompanied by 2,3-dimethylindole, the product of decarboxylation and rearrangement of **26**. The indolenine **17** can also be prepared by refluxing **23** in absolute alcoholic hydrogen chloride. This indicates that the transformation (**23** → **24**) in the Japp–Klingemann reaction can also be acid catalyzed.

We first examined the reaction of **17** with simple acid chlorides, such as acetyl chloride and chloroacetyl chloride, and found that when freshly purified reagents were employed, the Leuchs addition proceeded in high yield at room temperature. Interestingly, this is the first instance of addition of acyl chlorides to an indolenine-2-carboxylic acid derivative, the previous examples being limited to indolenine with 2-hydrogen, 2-methyl, or 2-phenyl substituents.[28-30] The indolenine **17** is less reactive than unconjugated ones, since benzoyl chloride could not be added. Reaction of **17** with ethoxycarbonyl chloride or benzyloxycarbonyl chloride was very slow, and trifluoroacetyl chloride did not react at all.

Two isomeric thioacetates, **28** and **29**, were isolated when potassium thioacetate was allowed to react with the product from acetyl chloride and **17** which had been allowed to warm to 40°, presumably as the result of a Plancher rearrangement[32] (**21** → **27**, Chart III). With

Chart III



potassium thiocyanate on **21**, the 2-isothiocyano compound **30** (Chart IV) was isolated instead of the expected 2-thiocyano compound.[33]

When **21** was dissolved in ethanol, it was rapidly converted to the ethyl ether **31**, a reaction analogous to the action of methanol on the reaction product from acetyl chloride and benzylidenemethylamine.[34]

When **32**, the product from chloroacetyl chloride and **17**, was allowed to react with thiourea, both chlorine atoms were displaced and a bisisothiouronium salt **33** resulted (Chart V). Ordinarily, chloroacetyl groups

(32) P. L. Julian, E. W. Meyer, and H. C. Printy, "Heterocyclic Compounds," Vol. 3, R. C. Elderfield, Ed., Wiley, New York, N. Y., 1952, Chapter 1.

(33) *Cf.* R. G. R. Bacon, "Organic Sulfur Compounds," Vol. I, N. Kharasch, Ed., Pergamon Press, New York, N. Y., 1961, p 308.

(34) H. Böhme and K. Hartke, *Chem. Ber.*, **96**, 600 (1963).

Chart IV

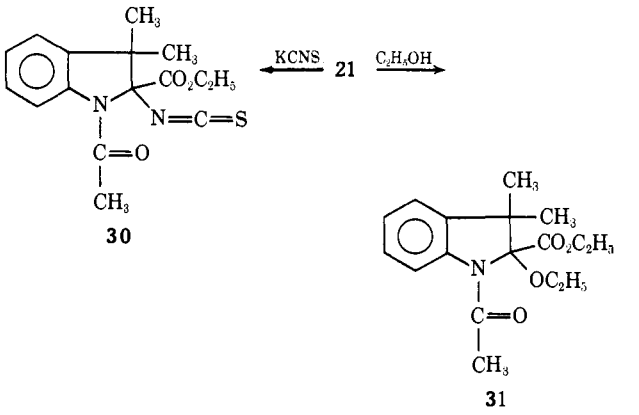
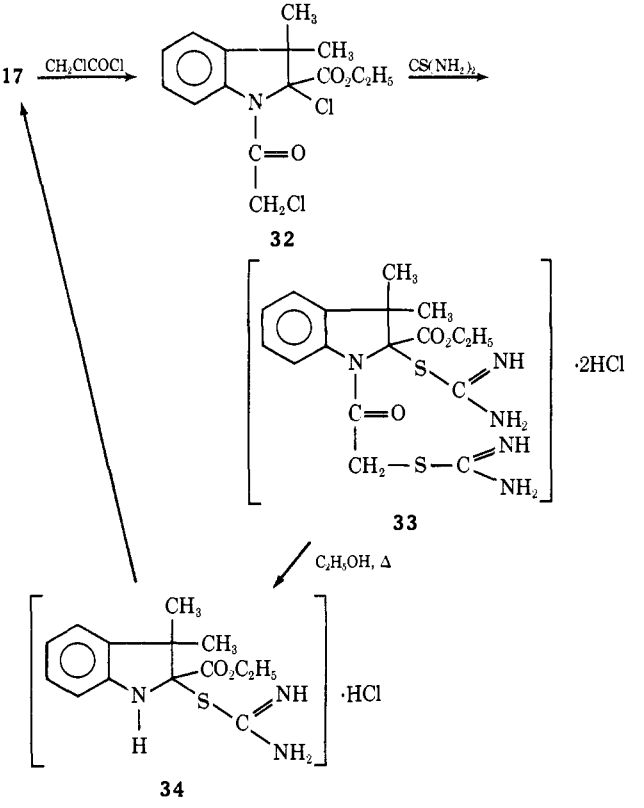


Chart V



are removed by thiourea in refluxing aqueous ethanol at pH 5 with the formation of pseudothiohydantoin.[35,36] Therefore, we expected the 2-isothioureido derivative **34**, but, under these conditions, isolated starting material **17**.

The pK_a of **33** was measured and found to be 7.7. When the solvolysis of **33** was attempted at pH 9.5 in the hope that **34** might be more stable as a neutral species, still only **17** was isolated. This suggests that unacetylated indoline-2-thiols are inherently unstable. Likewise, 2-indolinols are known only as *N*-acyl or *N*-alkyl derivatives.[32] At least these reactions prove that no Plancher rearrangement occurs *at room temperature* during acyl chloride additions or subsequent displacement reactions.

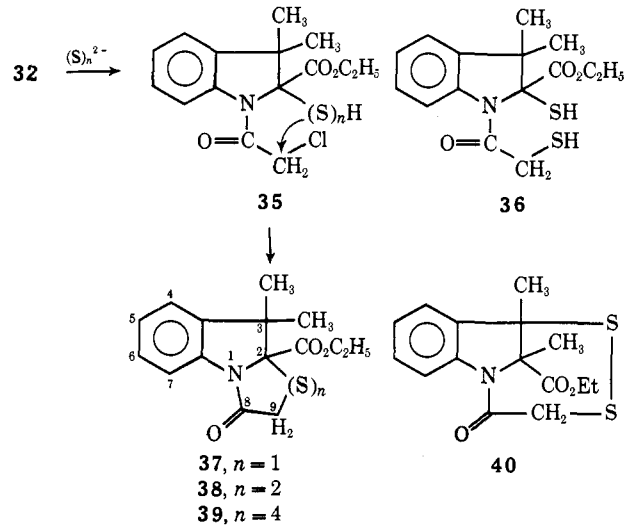
When **32** reacted with inorganic sulfides, such as ammonium sulfide, sodium mono-, di-, or tetrasulfide, or thiocarbonate, two products resulted: a mono- (**37**)

(35) A. Fontana and E. Scoffone, *Gazz. Chim. Ital.*, **98**, 1261 (1968).

(36) W. Steglich and H. G. Batz, *Angew. Chem.*, **83**, 83 (1971).

and a disulfide (**38**) in yields varying with the reactant (Chart VI). Sodium sulfide and sodium thiocarbonate

Chart VI



gave mainly the monosulfide **37** (*ca.* 40% yield), whereas ammonium sulfide and sodium di- and tetrasulfide, which all exist as mixtures of mono- and polysulfides, gave the mono- and disulfide in proportions of 2:1, 1:4, and 2:7, respectively.

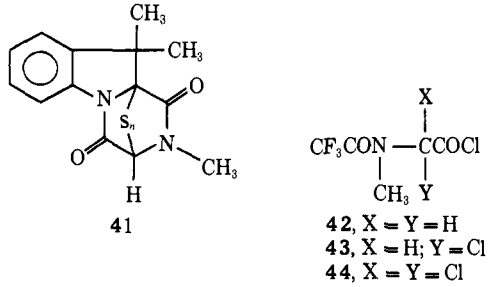
None of the thiol **36** could be detected; this together with the observation that sodium sulfide gives mainly (>90%) the monosulfide **37** suggests that **37** as well as **38** arise from an intramolecular displacement of chlorine in the sulfhydryl intermediate **35** (n = 1 or 2) and not *via* the dithiol **36**. A tetrasulfide **39** could not be detected although such a ring system forms easily in thio-bridged diketopiperazines.[23]

Models indicate that a cyclic sulfide is possible only in structures **37** and **38**. For the disulfide, but not the monosulfide, an alternate structure **40** may be envisaged. The disulfide **38**, however, could be converted quantitatively into the monosulfide **37** with triphenylphosphine[37] as evidence that no rearrangement occurred in the formation of the disulfide.

The nmr spectra of **37** and **38** show a surprisingly large difference in the δ value for the aromatic C_7 proton (δ 7.70 and 8.20, respectively), indicative of increased deshielding by the carbonyl group in **38**.

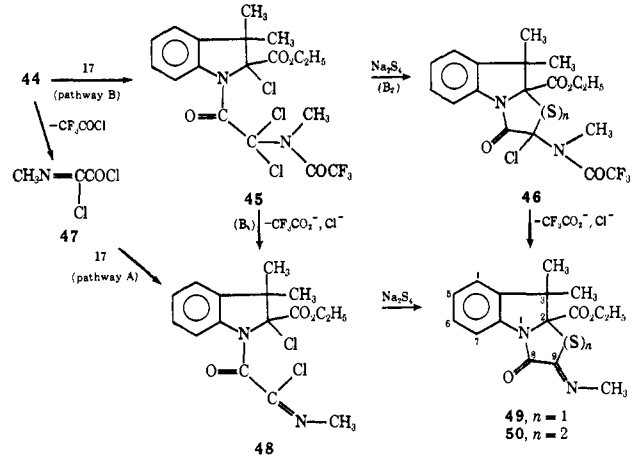
An N-acylated 9-amino analog of **38** on deacylation might undergo spontaneous ring closure and formation of the dithio-bridged diketopiperazine **41** (n = 2), an analog of dehydrogliotoxin (**12b**).

Accordingly, *N*-trifluoroacetylsarcosine chloride (**42**) was prepared from the free acid with thionyl chloride,[39] conditions mild enough not to affect the trifluoroacetyl group.[40] When **42** was refluxed in sulfuryl chloride in an attempt to prepare **43**, the α-dichloro acid chloride **44** was isolated. Details on this synthesis as well as some reactions of this interesting compound have been reported elsewhere.[41]



When the addition product from **44** and **17** was allowed to react with sodium tetrasulfide, a ninhydrin-positive, crystalline compound was isolated in 30% yield whose structure agrees with **49** (Chart VII).

Chart VII



As we have proposed elsewhere[41] **44** may decompose spontaneously to form **47**, which may then react with **17** to give **48** which in turn forms **49** with polysulfide ions in an intramolecular reaction (pathway A). Alternatively, pathway B proceeds *via* **45**, the addition product of **44** and **17**, which may then react in either or both of two ways: base-catalyzed hydrolysis of the *N*-trifluoroacetyl group to yield **49** *via* **48** (pathway B_1) or removal of the *N*-trifluoroacetyl group following reaction with polysulfide ions (pathway B_2). At the moment, we lack the definitive evidence necessary for a decision among these mechanistic possibilities.

We were unable to detect the disulfide **50**, possibly because it is either inherently unstable, or unable to survive the strongly alkaline conditions of the tetrasulfide reaction.[42]

The monosulfide **49** was reduced with sodium cyanoborohydride[44] to the amines **51** and **52** (Chart VIII), which are secogliotoxin analogs.

The course of the reduction is guided by steric induction of the carbethoxy group. The nmr spectrum of the reduction mixture showed two signals for the C_9 proton, at δ 5.65 and 5.29 in the ratio 2:1, respectively, and two signals at δ 2.54 with a separation of 2 Hz for the *N*-methyl group. It is assumed that the C_9 proton in the stereoisomer **52** is more shielded than in **51**, so that the signals at δ 5.65 and 5.29 can be assigned to structures **51** and **52**, respectively, of which **51** is the major (66%) and the diastereomer **52** the minor product (33%). An

(37) This method has been used to convert dehydrogliotoxin (**12b**)[9,38] and sporidesmin (**4**)[38] into monosulfides.

(38) S. Safe and A. Taylor, *J. Chem. Soc. C*, 1189 (1971).

(39) E. Schwenk and D. Papa, *J. Amer. Chem. Soc.*, **70**, 3626 (1948).

(40) F. Weygand, and U. Glockler, *Chem. Ber.*, **89**, 653 (1956).

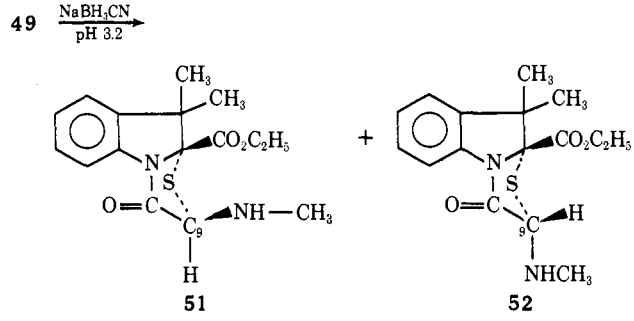
(41) H. C. J. Ottenheym, T. F. Spande, and B. Witkop, *J. Org. Chem.*, 37, 3358 (1972).

(42) The alkaline decomposition of organic disulfides very often produces monosulfides.[43]

(43) J. P. Danehy, *Int. J. Sulfur Chem., Part B*, **6**, 103 (1971).

(44) R. F. Borch, M. D. Bernstein, and D. Durst, *J. Amer. Chem. Soc.*, **93**, 2897 (1971).

Chart VIII

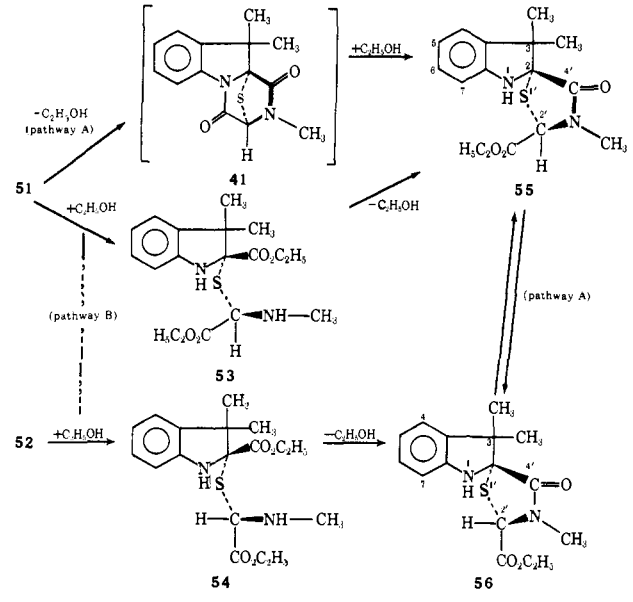


epithiodiketopiperazine can be formed only from **51** in which ester and amine functions are in a cis relationship. A bulkier hydride donor might make the reduction even more stereoselective.

Surprisingly the conversion of **49** into **51** and **52** led to no change in the ir spectrum of the amide carbonyl absorption (1705 cm^{-1}). This suggests that conjugation in $O{=}CC{=}NCH_3$ has little effect.

The mixture of monosulfides **51** and **52** was heated in ethanol in an attempt to form the epimonothiodiketopiperazine **41** (n = 1). Only in a sealed tube at 125° did a reaction occur yielding, besides starting material, a compound with a slightly higher R_f value on tlc.[45] This compound had nearly the same mass spectrum as the starting mixture, with differences only in peak intensities, indicative of closely related isomers of **51** and/or **52**. The nmr spectrum could best be interpreted as a mixture of structures **55** and **56** (Chart IX),

Chart IX



compounds derived from the starting material by an interesting translactamization.

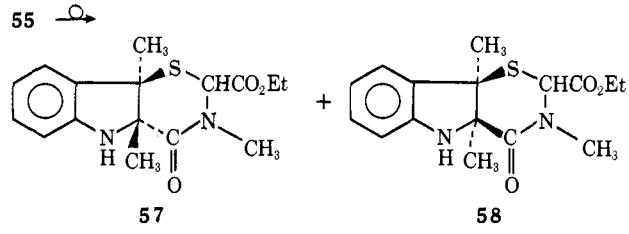
The two signals assigned to the N-methyl groups were shifted downfield (δ 3.23 and 3.13) and show a larger difference in chemical shifts than in **51** and **52** ($\Delta\delta$ = 5 and 2 Hz, respectively). Surprisingly only one broad signal was observable for the C_2' proton. Therefore, the possibility that we had in hand only one pair of enantiomers, **55** or **56**, had also to be considered; the two signals for the N-methyl group could be explained by a conformational or long-range coupling effect.

(45) Under these conditions compound **37** was found to be stable.

However, nmr spectra at −20 or −40° and irradiation of the C_2' proton failed to change the relative intensities of the two N-methyl signals and indicated that the isolated material was most likely a mixture of two pairs of enantiomers, **55** and **56**. An nmr of the recovered starting material mixture indicated that the proportion of **52** in the mixture had increased greatly and was now twice that of **51**.

Particular attention was given to these considerations, for if only one pair of enantiomers had been formed, this probably would have been **55**, derived only from the reactive starting material **51**, *via* the desired diketopiperazine **41** (pathway A, Chart IX). A Dreiding model shows that the epimonothiodiketopiperazine ring system in **41** is a highly strained though not an impossible one as has been shown by Taylor.[9,38] The occurrence of two pairs of enantiomers could then be explained by epimerization at C_2' in **55**. A deuterium-exchange study is planned to check this possibility.

The occurrence of **55** and **56** would also be explained by pathway B, Chart IX. If the amide groups in **51** and **52** were cleaved by ethanol, the α-thio-bridged α-amino acid esters **53** and **54** would result. These could lactamize in two ways, yielding besides the starting materials the structures **55** and **56**, respectively. Structures **53** and **54** with an unacylated α-thio amino acid moiety are undoubtedly unstable[46] (see also Chart V and accompanying text), and should break down to the indolenine ester **17**. However, the reaction mixture **51** + **52** ⇄ **55** + **56** showed only two spots on tlc with no trace of side products,[47] making this mechanism unlikely.



Milder reaction temperatures and the use of nonprotic solvents provided no new information. At 90° the formation of the new isomers is very slow and no new component could be detected; diglyme as solvent at 90 or 120° failed to give any identifiable product. At present there is no evidence permitting a choice between pathways A and B.

Experimental Section

Infrared spectra were measured with Perkin-Elmer spectrophotometers, Models 237B ($CHCl_3$ or CCl_4) and 421 (KBr), and uv spectra with a Cary Model 11 (95% EtOH). Mass spectra were obtained with the double-focusing Hitachi RMU-6E mass spectrometer. Proton magnetic resonance spectra were measured on the Varian Associates Model A-60 spectrometer. Chemical shifts are reported as δ values (ppm) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated

(46) Pojer and Rae[27] described the synthesis of **13** and 2,2′-dibenzamido-2,2′-dithiodipropanoic acid, in which the amino function is acylated. Interestingly, the deacylated products were not mentioned.

(47) A Plancher rearrangement[32] producing **57** and **58** cannot be completely ruled out. The shift in amide carbonyl absorption from 1690 to 1640 cm^{-1} which accompanies this reaction is somewhat unexpected. Although the latter absorption is normal for a tertiary amide, it could also indicate that the amide is part of a six-membered ring. Arguing against this possibility, however, is the similarity of the 3-methyl signals in the nmr spectra of starting materials and products.

otherwise. Melting points were taken on a Kofler hot stage and are corrected. Thin layer chromatography (tlc) was carried out using Merck precoated silica gel F-254 plates (thickness: 0.25 mm for analytical, 2.0 mm for preparative); spots were visualized with a uv hand lamp, iodine vapor, or a 0.1% solution of ninhydrin in methanol–1-butanol–2 *N* acetic acid (20:10:1 v/v).

Ethyl α-Isopropylacetoacetate (22). This compound was prepared from ethyl acetoacetate and 2-bromopropane following the procedure for the synthesis of ethyl *n*-propylacetoacetate.[48] Vacuum distillation (34 mm) on a Vigreux column yielded two fractions, bp 83–105° and 105–108°, the latter being the desired compound, the former being the O-alkylated product.[49] When freshly distilled ethyl acetoacetate was used, only the desired **22** resulted in 40% yield: nmr δ 4.14 (q, 2 H, CH_2CH_3), 3.22 (d, 1 H, COCHCO, J = 9 Hz), 2.40 (mult, 1 H, CH_3CHCH_3), 2.15 (s, 3 H, CH_3CO), 1.21 (t, 3 H, CH_2CH_3), 0.9 (2 d, 6 H, CH_3CHCH_3, J = 6 Hz, spacing 2 Hz). O-Alkylated product: δ 4.12 (q, 2 H, CH_2CH_3), 3.45 (s, 1 H, C=CH), 2.18 (s, 3 H, $CH_3C{=}C$), 1.21 (t, 3 H, CH_2CH_3), 0.90 (2 d, 6 H, CH_3CHCH_3, J = 6 Hz, spacing 2 Hz).

Ethyl α-Phenylazo-α-isopropylacetoacetate (23). Solution A. A solution of 51.0 g (0.30 mol) of ethyl isopropylacetoacetate (**22**) in 200 ml of ethanol was cooled at −15° (ice–salt bath). Just before the addition of solution B to A, 232.5 ml of 5 *N* NaOH (1.15 mol), cooled to −15°, was added at once.

Solution B. A solution of benzenediazonium chloride was prepared from 27.9 g (0.30 mol) of freshly distilled aniline, 255 ml (1.02 mol) of 4 *N* HCl, and 21.0 g (0.30 mol) of sodium nitrite dissolved in 50 ml of water. All solutions were cooled at −15°. Immediately after the addition of the sodium nitrite solution, solution B was added with swirling to solution A and cooled in a Dry Ice–acetone bath. After the solution was stirred for 45 min at −15°, an oil separated from the dark red colored reaction mixture. After acidification with 2 *N* HCl, this oil was extracted with ether. The organic layer was washed with water, 5% $NaHCO_3$, and finally water until neutral, dried (Na_2SO_4), and filtered, and the ether was removed to yield a dark red residue. From this oil, 16.7 g (0.098 mol) of unreacted **22** could be isolated by vacuum distillation (bp 48–52° (0.5 mm)). The dark red residue (48.0 g, 0.175 mol, 87% based upon recovered starting material) was stored at room temperature: nmr, δ 7.5 (mult, 5 H, C_6H_5), 4.28 (q, 2 H, CH_2CH_3), 2.75 (mult, 1 H, CH_3CHCH_3), 2.17 (s, 3 H, CH_3CO), 1.28 (t, 3 H, CH_2CH_3), 1.10 (d, 6 H, CH_3CHCH_3); uv λ_{max}^{EtOH} 277 nm (shoulders at 215 and 330 nm).

Ethyl α-Keto-β-methylbutyrate Phenylhydrazone (24). To a solution of 48.0 g (0.175 mol) of the azo compound **23** in 250 ml of ethanol was added 50 ml of concentrated aqueous NH_4OH. The mixture was stirred and kept at 50° while the reaction was monitored by uv spectroscopy (shift from λ_{max} 277 to 325 nm). The reaction was stopped after the starting material had disappeared (45 min). The volume was reduced until *ca.* 100 ml, water was added, and the dark red solution was extracted with ether after acidification with 6 *N* HCl. The organic layer was washed with water until neutral and dried (Na_2SO_4), and the ether was removed to yield 38.5 g (0.163 mol, 93%) of a dark red solid, which was used without further purification in the next step: uv λ_{max}^{EtOH} 323 nm, shoulder at 292 nm; nmr δ 7.18 (broad mult, 6 H, C_6H_5 + NH), 4.26 (q, 2 H, CH_2CH_3), 3.02 (heptet, 1 H, CH), 1.35 (t, 3 H, CH_2CH_3), 1.27 (d, 6 H, CH_3CHCH_3).

A small sample was purified by vacuum sublimation at 90° (0.75 mm), followed by crystallization from hexane and washing with petroleum ether, to give thin needles, mp 100.5–101.5°; mass spectrum (175°) *m/e* 234 (M^+), 219 ($M^+ - CH_3$), 199, 191 ($M^+ - C_3H_7$), 189 ($M^+ - OC_2H_5$), 160 ($M^+ - 1 - CO_2 - C_2H_5$), 145 (160 − CH_3), 105, 92 (aniline − 1).

α-Keto-β-methylbutyric Acid Phenylhydrazone (25). This compound was prepared[31] in 20% yield and showed on tlc (3% acetic acid–benzene) only one spot: uv λ_{max}^{EtOH} 341 nm, shoulders at 297, 288 nm; nmr δ 7.3 (mult, 6 H, C_6H_5 + NH), 3.08 (heptet, 1 H, CH_3CHCH_3, J = 7.5 Hz), 1.22 (d, 6 H, CH_3CHCH_3). Other properties agreed well with reported values.

Ethyl 3,3-Dimethylindolenine-2-carboxylate (17). An ice-cooled solution of 12.0 g (51 mmol) of the crude hydrazone ester **24** in 100 ml of absolute ethanol was saturated with dry HCl (solution changes from dark brown to violet in color) and then refluxed for 9 min during which time ammonium chloride precipitated. The ethanol was removed and the residue extracted with ether and 5% Na_2CO_3. The aqueous layer did not contain any free acid **26**. The organic layer was washed with water until neutral and dried (Na_2SO_4) and the ether removed to yield 10.4 g (48 mmol, 94%) of a dark brown solid, which was purified by vacuum sublimation at 90° (1.0 mm), followed by recrystallization from petroleum ether (80–100°) at −20°. Large colorless needles with mp 78–79° (lit.[31] mp 79–80°) were obtained in 52% yield: tlc (2% ethanol–benzene) one spot; uv λ_{max}^{EtOH} 294 and 232 nm (equal intensities); ir (KBr) 3070, 3050, 2980, 2950, 2870, 1713 (CO), 1542, 1460, 1365, 1335, 1310, 1280, 1210, 1190, 1120, 1090, 1065, 1010, 860, 785, 770, and 750 cm^{-1}; nmr δ 7.8 (mult, 1 H, C_7-H), 7.35 (mult, 3 H, C_{4-6}-H), 4.46 (q, 2 H, CH_2CH_3), 1.52 (s, 6H, CH_3CCH_3), 1.43 (t, 3 H, CH_2CH_3); mass spectrum (175°), *m/e* 217 (M^+), 202 ($M^+ - CH_3$), 188, 173, 172 ($M^+ - OC_2H_5$), 158, 145, 144 ($M^+ - CO_2C_2H_5$), 143, 130, 128, 117, 115, 103, 91, 77.

The ester **17** was also prepared according to Robinson and Sugimome[31] from the hydrazone acid **25**, yielding 6.5% of the ester **17** and 35.5% of the acid **26**.

A 2.0-g sample of the azo ester **23** was converted into **17** in 48% yield by the treatment given to the hydrazone ester **24** mentioned above. Both procedures led to material which was found to be identical in all respects with the specimen previously obtained.

Ethyl 1-Acetyl-2-thioacetyl-3,3-dimethylindoline-2-carboxylate and Isomer (28 and 29). A solution of **17** (434 mg, 2 mmol) in 4 ml of freshly distilled acetyl chloride was kept at 40° for 15 hr, during which time the reaction was monitored by tlc (4% ethanol–benzene). After addition of 5 ml of benzene, solvent and excess reagent were removed under vacuum and exclusion of moisture. Tlc (4% ethanol–benzene) showed two spots with R_f values smaller than the starting material: ir ($CHCl_3$) 1750, 1690 cm^{-1}, disappearance of 1713 cm^{-1}; uv λ_{max}^{EtOH} 248 nm, shoulders at 278 and 286 nm (spectrum is actually of the ether **31**). To the light brown oil was added an alcoholic solution of 570 mg (5 mmol) of potassium thioacetate after which potassium chloride separated. Stirring was continued for 2 hr; the alcohol was removed and the residue extracted with ether. The ether layer was washed with 5% $NaHCO_3$ and water until neutral and dried (Na_2SO_4), and the ether was removed to yield 545 mg of a yellow oil: tlc (4% ethanol–benzene) besides starting material, two spots with lower R_f. The oil was chromatographed on a silica gel column (100 g) with 4% ethanol–benzene, yielding three fractions: the first fraction appeared to be starting material **17** (30 mg), the second and third were assigned structures **29** and **28**, respectively, on the following basis. Ir, identical for both fractions ($CHCl_3$) 1750 (broad ester) and 1680 cm^{-1} (broad, CON + COS). Fraction 2: nmr δ 7.4–7.0 (mult, 4 H, C_6H_4), 4.30 (q, 2 H, CH_2CH_3), 2.48 (s, 3 H, CH_3CON), 2.32 (s, 3 H, CH_3COS), 1.47 (s, 3 H, $C_3C_\alpha H_3$), 1.37 (s, 3 H, $C_3C_\beta H_3$), 1.20 (t, 3 H, CH_2CH_3); mass spectrum (190°) *m/e* 335 (M^+), 305 ($M^+ - C_2H_6$), 293 ($M^+ - CH_3CO$), 260 ($M^+ - CH_3COS$), 217 ($M^+ - CH_3CO - CH_3COS$). Fraction 3: nmr δ 7.40–7.0 (mult, 4 H, C_6H_4), 4.25 (q, 2 H, CH_2CH_3), 2.48 (s, 3 H, CH_3CON), 2.18 (s, 3 H, CH_3COS), 1.60 (s, 3 H, $C_3 - C_\alpha H_3$), 1.28 (s, 3 H, $C_2C_\beta H_3$), 1.20 (t, 3 H, CH_2CH_3); mass spectrum, nearly identical with that of fraction 2. When the reaction of the acid chloride and **17** was carried out at room temperature, only one spot was observed in tlc, which had the same R_f value as fraction 3.

Ethyl 1-Acetyl-2-isothiocyano-3,3-dimethylindoline-2-carboxylate (30). A solution of **17** (10 mg, 0.46 mmol) in 2 ml of dry benzene was allowed to react with acetyl chloride (50 mg) and stirred at room temperature for 15 hr. After that time no starting material could be detected on tlc (4% ethanol–benzene, R_f 0.24). The solvent and excess acetyl chloride were removed *in vacuo* with exclusion of moisture. To the light brown oil was added with stirring an alcoholic solution of 40 mg of potassium thiocyanate after which potassium chloride separated. Stirring was continued for 15 min, the solvent removed, and the residue extracted with water–ether. The ether layer was washed twice with water and dried (Na_2SO_4), and the solvent was removed to yield 12 mg (0.38 mmol, 83%) of a light yellow oil: tlc (4% ethanol–benzene) showed only one spot, R_f 0.45; ir ($CHCl_3$) 2980 (broad), 2040 (strong, broad, $-N{=}C{=}S$[33]), 1760 (ester), 1680 cm^{-1} (CH_3CON); mass spectrum (150°) *m/e* 318 (M^+), 305, 278, 260 ($M^+ - NCS$), 244, 232, 217.

Ethyl 1-Acetyl-2-ethoxy-3,3-dimethylindoline-2-carboxylate (31). The addition product **21** of 434 mg (2 mmol) of **17** and 3.4 g (43 mmol) of acetyl chloride was prepared as described above for the preparation of **30**. To the light brown oil was added 5 ml of absolute ethanol; the solution was stirred for 2 hr at room temperature after which excess reagent was removed, yielding 470 mg of a yellow oil: tlc (4% ethanol–benzene) showed only one spot, R_f 0.20; nmr δ 7.8 (mult, 1 H, C_7-H), 7.4–7.0 (mult, 3 H, C_{4-6}-H), 4.30

(48) A. I. Vogel, "A Textbook of Practical Organic Chemistry," 3rd ed, Longmans, Green and Co., New York, N. Y., 1957, p 481.

(49) S. T. Yoffe, E. I. Fedin, P. V. Petrovskii, and M. I. Kabachnik, *Tetrahedron Lett.*, 2661 (1966).

(q, 2 H, $COOCH_2$–), 3.30 (q, 2 H, $COCH_2$–), 2.35 (s, 3 H, CH_3CON), 1.45 (s, 6 H, CH_3CCH_3), 1.32 (t, 3 H, $-COOCH_2CH_3$), 1.13 (t, 3 H, $COCH_2CH_3$); mass spectrum (160°) *m/e* 305 (M^+), 275 ($M^+ - C_2H_6$), 232 (275 − CH_3CO), 218, 217, etc.

2,9-Bisisothiouronium-1-acetyl-2-carbethoxy-3,3-dimethylindoline Dichloride (33). The addition product of **17** (434 mg, 2 mmol) and 3.3 g (26 mmol) of α-chloroacetyl chloride in 3 ml of dry benzene was prepared as described for the preparation of **30** (stirred for 15 hr). To the light yellow oil was added under stirring a solution of 381 mg (5 mmol) of thiourea in 10 ml of 2-propanol. Stirring was continued for 15 min, after which the clear solution was refluxed for 1 hr. Within a few minutes a precipitate formed. The reaction mixture was then cooled in ice, the precipitate filtered off, and from the filtrate the solvent removed to yield 950 mg of a yellow powder: uv λ_{max}^{EtOH} 247 nm (shoulders at 278 and 286 nm); $pK_a = 7.7$; nmr δ 7.67 (mult, 1 H, C_7-H), 7.48 (mult, 3 H, C_{4-6}-H), 4.44 (q, 2 H, CH_2CH_3), 3.31 (s, 2 H, $-COCH_2S$–), 1.50 (s, 6 H, CH_3CCH_3), 1.42 (t, 3 H, $-CH_2CH_3$).

Conversion of 33 into 17. The yellow powder (100 mg) was dissolved in 10 ml of 40% ethanol; the pH of this solution was adjusted at 5.2 or in another experiment to 9.5 with 0.5 *M* sodium acetate and 5% $NaHCO_3$, respectively. The reaction mixtures were refluxed for 5 min, then the volume was reduced to 5 ml, and the mixtures were extracted with ethyl acetate. The organic layers were dried (Na_2SO_4) and the solvent was removed, yielding 27 and 28 mg (59% and 60%, respectively) of crystalline material having R_f values, uv, and ir spectra identical with those of **17**.

2-Carbethoxy-3,3-dimethylindolino[2,1-*b*]thiazolidinone-8 (37 and 38).[50] The addition product **32** of 1.085 g (5 mmol) of **17** and 6.0 g (40.7 mmol) of freshly distilled α-chloroacetyl chloride in 10 ml of dry benzene was prepared as described for the preparation of **30** (stirred for 15 hr). A solution of the light yellow oil in 25 ml of dry diglyme was divided into five 5-ml aliquots. To the first sample was added quickly with ice cooling 20 ml of a freshly prepared, ice-cold solution of sodium disulfide (1.1 *M*) in water. The latter was prepared the following way: 3.4 g (0.11 mol) of powdered sulfur was dissolved in a solution of 26 g (0.11 mol) of $Na_2S \cdot 9H_2O$ in *ca.* 70 ml of water by warming and stirring the wine-red mixture. The clear solution was then diluted to 100 ml. To minimize saponification, the reaction time was kept less than 5 min. The reaction was stopped by acidification with 2 *N* HCl. The mixture was extracted with ethyl acetate, and the organic layer was filtered (to remove S_8), washed with water until neutral, and dried (Na_2SO_4). The solvents were removed under high vacuum to yield 313 mg of a yellow oil, which showed on tlc (4% ethanol–benzene) two spots with R_f values of 0.48 and 0.55, respectively. Two fractions (24 mg, 8%, and 103 mg, 32%) could be isolated by preparative tlc (developed one time with 3% ethanol–benzene and one time with 2% ethanol–benzene). The product with lower R_f value was found to be the monosulfide **37**; the other, the disulfide **38**. Both compounds could be further purified by vacuum sublimation (120° (0.7 mm)) followed by recrystallization from ethanol–hexane for **37** (mp 103.5–104.5°] and from ethanol–water for **38** (mp 111–113°).

Monosulfide 37: ir ($CHCl_3$) 2980, 1730 (ester), 1685 (amide), 1600, 1592, 1480, 1455, 1390, 1365, 1285, 1260, 1145, 1105, and 1025 cm^{-1}; nmr δ 7.70 (mult, 1 H, C_7–H), 7.18 (mult, 3 H, C_{4-6}–H), 4.18, (q, 2 H, CH_2CH_3), 4.07 (d, 1 H, C_9–H_α, $J_{\alpha\beta}$ = 15 Hz, AB spectrum), 3.70 (d, 1 H, C_9–H_β), 1.45 (s, 3 H, C_3–$C_\alpha H_3$), 1.35 (s, 3 H, C_3–$C_\beta H_3$), 1.21 (t, 3 H, CH_2CH_3); mass spectrum (180°), *m/e* 291 (M^+), 218 ($M^+ - CO_2C_2H_5$), 203, 158, 145, 144.

Anal. Calcd for $C_{15}H_{17}NO_3S$: C, 61.83; H, 5.88; N, 4.81; S, 11.00. Found: C, 61.73; H, 5.82; N, 4.54; S, 10.76.

Disulfide 38: ir ($CHCl_3$) 2980, 1732 (ester), 1650 (amide), 1595, 1475, 1455, 1390, 1368, 1284, 1130, 1100, and 1025 cm^{-1}; nmr δ 8.20 (mult, 1 H, C_7–H), 7.15 (mult, 3 H, C_{4-6}-H), 4.18 (q, 2 H, CH_2CH_3), 4.03 (d, 1 H, C_9–H_α, $J_{\alpha\beta}$ = 17 Hz, AB spectrum), 3.49 (d, 1 H, C_9–H_β), 1.50 (s, 3 H, C_3–$C_\alpha H_3$), 1.34 (s, 3 H, C_3–$C_\beta H_3$), 1.20 (t, 3 H, CH_2CH_3); mass spectrum (180°), *m/e* 323 (M^+), 291 ($M^+ - S$), 259 ($M^+ - S_2$), 252, 250 ($M^+ - CO_2C_2H_5$), 218 (base peak), 203, 172, 158, 145, 144.

Anal. Calcd for $C_{15}H_{17}NO_3S_2$: C, 55.70; H, 5.30; N, 4.33; S, 19.82. Found: C, 55.93; H, 5.17; N, 4.39; S, 19.50.

One 5-ml aliquot was treated with 20 ml of an aqueous ammonium sulfide solution (7%). Reaction conditions and work-up were the same as described above. After preparative tlc, 80 mg (27%) of monosulfide **37** andd 40 mg (12.4%) of disulfide **38** were isolated.

Two other 5-ml aliquots were allowed to react with an aqueous solution of sodium monosulfide (20 ml of a 1.1 *M* solution), or freshly prepared sodium thiocarbonate[61] (20 ml of a 1.1 *M* solution), respectively. Reaction conditions and work-up were as described above. Crude material was purified by vacuum sublimation (110° (0.7 mm)) (material that sublimed at a lower temperature was found to be starting material **17**) and recrystallization from ethanol–water to yield 120 (40%) and 123 mg (42%), respectively, of the monosulfide **37**. Tlc of the crude material showed only traces of the disulfide **38**.

The last aliquot was allowed to react with 20 ml of a 1.1 *M* aqueous solution of freshly prepared sodium tetrasulfide. The latter was prepared analogous to the preparation of Na_2S_2 from 10.2 g (0.33 mol) of powdered sulfur and 26 g (0.11 mol) of $Na_2S \cdot 9H_2O$. Reaction conditions and work-up were the same as described above. After preparative tlc, 42 mg (14%) of **37** and 154 mg (47%) of **38** could be isolated besides 26 mg (12%) of the starting material **17**.

Conversion of Disulfide 38 into Monosulfide 37. A solution of 13 mg (4.1×10^{-2} mmol) of **38** and 22 mg (8.4×10^{-2} mmol) of triphenylphosphine in 1 ml of absolute ethanol was kept, wrapped in aluminum foil, at room temperature for 20 days. The rate of reaction was monitored by tlc. The solvent was removed and the residue subjected to preparative tlc (developed two times, 3% ethanol–benzene), to yield 9.5 mg (3.25×10^{-2} mmol, 80%) of **37**. Identification was based upon mass spectrometric and ir data.

***N*-Trifluoroacetyl-α,α-dichlorosarcosyl Chloride (44).** The synthesis of this compound (bp 54–58° (40 nm)) is described elsewhere.[41]

2-Carbethoxy-3,3-dimethyl-9-methyliminoindolino[2,1-*b*]thiazolidinone-8 (49). The addition product of 1.52 g (7 mmol) of **17** and 3.8 g (14 mmol) of **44** in 25 ml of dry benzene was prepared as described for the preparation of **30** (stirred for 16 hr) and yielded 3.86 g of a light yellow oil. To an ice-cold solution of this oil in 30 ml of dry diglyme was added quickly an ice-cold, freshly prepared aqueous solution of sodium tetrasulfide (40 ml, 44 mmol, prepared as described for the synthesis of **37** and **38**). The reaction mixture was kept at 0° and stirred for 5 min, after which it was extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and the solvent removed to yield 1.94 g of a dark brown oil; 0.65 g of this oil was subjected to preparation tlc on five plates (developed two times with 4% ethanol–benzene) to yield 192 mg (0.88 mmol, 38%) of starting material and 270 mg of a still impure, ninhydrin-positive material. The latter was rechromatographed on five plates as before yielding 220 mg (0.7 mmol, 30%) of crystalline material (ninhydrin-positive) which could be further purified by vacuum sublimation at 100° (0.5 mm): tlc (5% ethanol–benzene), one spot R_f 0.41. A small sample was crystallized from slowly evaporating chloroform: mp 101–103°; ir ($CHCl_3$) 2960, 1730 (ester), 1705 (amide), 1645 (methylimino), 1475, 1455, 1390, and 1290 cm^{-1}; nmr δ 7.88 (mult, 1 H, C_7–H), 7.22 (mult, 3 H, C_{4-6}–H), 4.15 (q, 2 H, CH_2CH_3), 3.46 (s, 3 H, NCH_3), 1.51 (s, 3 H, C_3–$C_\alpha H_3$), 1.23 (s, 3 H, C_3–$C_\beta H_3$), 1.18 (t, 3 H, CH_2CH_3); mass spectrum (160°), *m/e* 318 (M^+), 302 ($M^+ - CH_4$), 274 ($M^+ - OC_2H_5$), 259 (274 − CH_3), 245 ($M^+ - CO_2C_2H_5$, base peak), 230 (245 − CH_3), 218, 204, 202, etc.

2-Carbethoxy-3,3-dimethyl-9-methylaminoindolino[2,1-*b*]thiazolidinone-8 (51 and 52). To a solution of 285 mg (0.9 mmol) of the Schiffs base **49** in 5 ml of absolute ethanol at room temperature was added a trace of bromocresol green; 2 *N* methanolic HCl was added until the indicator turned yellow, and 200 mg (3.2 mmol) of sodium cyanoborohydride was added with stirring. Additional HCl–methanol solution was added to maintain the yellow color. Stirring was continued for 10 min. The solution was poured into 50 ml of ice-cold 0.1 *N* NaOH, saturated with NaCl, and extracted twice with ice-cold ethyl acetate. The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to give 288 mg (0.9 mmol, 100%) of a colorless oil, which showed only one spot on tlc (5% ethanol–benzene, R_f 0.38, ninhydrin-positive): ir ($CHCl_3$) 3350 (weak, broad, NH), 2980, 2940, 2800, 1730 (ester), 1705 (amide), 1605, 1590, 1480, 1460, 1395, 1370, 1290, and 1265 cm^{-1}. nmr δ 7.81 (mult, 1 H, C_7–H), 7.21 (mult, 3 H, C_{4-6}–H), 5.65 (s, $^2/_3$ H, C_9–H of **51**), 5.29 (s, $^1/_3$ H, C_9–H of **52**), 4.19 (q, 2 H, CH_2CH_3), 2.54 (2 singlets, separation 1.5 Hz, 3 H, NCH_3), 1.49, 1.34, and 1.25 (overlapping singlets, C_3–CH_3 in 51 and 52), 1.34 and 1.22 (2 t, CH_2CH_3 of **51** and **52**), 1.92 (broad, 1 H, NH); mass spectrum (160°) *m/e* 320 (M^+), 279, 275 ($M^+ - OC_2H_5$), 264, 247 ($M^+ - CO_2C_2H_5$), 245, 234, 230, 219, 218, 202, 192, 178, 175, 149, 146 (base peak).

Rearrangement of 51 into 55 and 56. A solution of 40 mg (0.126

(50) According to IUPAC rules the nomenclature for **37** and **38** should be 2,3,9,9a-tetrahydro-3-keto-9,9-dimethyl-9a-carbethoxythiazolo[3,2-*a*]indole and 3,4,5,6,10,10a-hexahydro-4-keto-10,10-dimethyl-10a-carbethoxy-1,2,5-dithiazino[5,6-*a*]indole, respectively. For convenience we use the names above.

(51) D. J. Martin and C. C. Greco, *J. Org. Chem.*, 33, 1275 (1968).

mmol) of the mixture of **51** and **52** in 5 ml of absolute ethanol was heated in a sealed ampoule at 108° for 24 hr and then at 125° for 16 hr. Tlc (6% ethanol–benzene) showed the presence of only two products, the starting material and a product with larger R_f. The solvent was removed and the brown oily residue subjected to preparative tlc (developed three times with 5% ethanol–benzene), to yield 26 mg (65%) of "starting material" and 14 mg (35%) of isomerized product: tlc (6% ethanol–benzene) only one spot, R_f 0.50; ir ($CHCl_3$) 3400 (sharp, NH), 2980, 2940, 2860, 1730 (ester), 1640 (amide), 1600, 1525, 1480, 1460, 1395, and 1370 cm^{-1}; nmr δ 8.15 (mult, 1 H, C_7–H), 7.20 (mult, 3 H, C_{4-6}–H), 5.50 (broad singlet, 1 H, C_2'–H), 4.17 (q, 2 H, CH_2CH_3), 3.23 and 3.13 (2 singlets, separated 5 Hz, 3 H, N–CH_3), 2.0 (broad S, 1 H, NH), 1.47 and 1.29 (2 singlets, 6 H, CH_3CCH_3), 1.29 (t, 3 H, CH_2CH_3); mass spectrum (160°), *m/e* 320 (M^+), identical with that for **51** and **52**, except for a stronger signal at *m/e* 304 ($M^+ - CH_4$) and a weaker one at *m/e* 247 ($M^+ - CO_2C_2H_5$) and 245.

The nmr spectrum of the isolated "starting material" showed a change in that the ratio of the two signals from the C_9-proton was reversed (now δ 5.65/5.29 = 1:2), indicating that only the cis enantiomers **51** have been isomerized.

Acknowledgment. We are indebted to Professor R. J. F. Nivard for reading the manuscript.

Reduction of Δ^{24} of Lanosterol in the Biosynthesis of Cholesterol by Rat Liver Enzymes. II. Stereochemistry of Addition of the C-25 Proton

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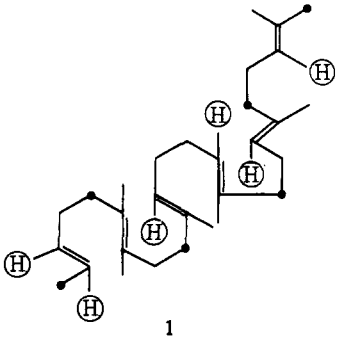
Abstract: *Mycobacterium smegmatis* converts cholesterol to (25*S*)-26-hydroxycholest-4-en-3-one. It was shown, with the use of [$^{14}C_5$]cholesterol biosynthesized from [2-^{14}C]mevalonic acid in the S-10 fraction of rat livers, that the 26-^{14}C atom bore the oxygen atom. Evidence is provided that the 26-hydroxylation by *M. smegmatis* proceeds without loss of the C-25 hydrogen. Barring rearrangement and migration of this hydrogen, it may be inferred that the C-26-hydroxylation proceeds without epimerization at C-25. Previously, we demonstrated that in the reduction of Δ^{24} of lanosterol a 24-*pro-S* hydrogen is added. It follows that the reduction of Δ^{24} of lanosterol in the biosynthesis of cholesterol in the S-10 fraction of rat liver entails the overall cis addition of two hydrogen atoms.

It is now well established that the biosynthesis of squalene (**1**) proceeds with the incorporation of six

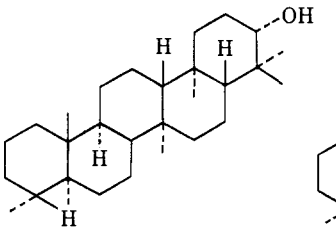


1

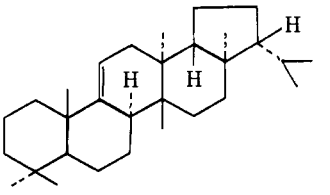
•, derived from C-2 of MVA
Ⓗ, derived from 4-*pro-R* hydrogen of MVA

C-2 carbon atoms of mevalonic acid[2] (MVA) and six 4-*pro-R* hydrogen atoms of MVA. The location of the C-2 and 4-*pro* H atoms of MVA in squalene is as shown in **1**. The terminal methyls of squalene are derived from C-2 and C-3′ carbons of MVA. The cis geometry of the methyl derived from C-2 of MVA and of the hydrogen on *each* of the terminal double bonds of **1** has been proven.[3]

The operation of both oxidative and proton-initiated mechanisms of enzymatic cyclization of squalene are now firmly established. The 3-deoxytriterpenes are thought to be formed *via* a nonoxidative proton initiated attack on a terminal double bond of squalene.[4,5] This mechanism was shown to operate in the biosynthesis of the pentacyclic triterpene tetrahymanol (**2**) in the proto-



2



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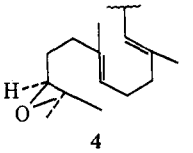

4

zoan *T. pyriformis*. The overall process is equivalent to the acquisition by squalene of the elements of water (*loc. cit.*). It seems likely that in the fern *Oleoandra*

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