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-poly sulfide mixture gave a monosulfide 49 (30% yield) but no disulfide 50. Reduction of 49 with NaCNBH3 proceeded stereoselectively to afford mainly the secogliotoxin analog 51 in addition to the diastereoisomer 52. Cyclization of this mixture presumably led to the strained epimonomothiodiketopiperazin e 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 (10-11)16 which have no antibacterial activity. 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 (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 anti- viral activity.

Two other fungal metabolites, chetomin (C6H3N2O3S)19 and oryzachlorin (C66H6N2O3S2Cl),20 of unknown structure, probably contain the epidithiodiketo- piperezine ring. The former is active against gram-positive bacteria19 and viruses21 while the latter has only antifungal and antiviral activity.20

The mechanism of antiviral action of gliotoxin22 and aranotin13 depends upon the specific inhibition of RNA-dependent RNA polymerases from tumor-producing viruses or blocking of the synthesis of viral RNA21 in the case of chetomin. Several syntheses of simple epidithiodiketopiperazines have been reported,21,13,19-25 which feature the addition of sulfur substituents to a preformed diketopiperazine system. Surprisingly, the simple model 1a is highly active in inhibiting viral RNA synthesis,23 in support of the view26 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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The reaction of sulfur nucleophiles (e.g., SCOCH₃⁻, SCN⁻, S₂O₃²⁻, 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.

The azo ester 23, when prepared from ethyl α-isopropylacetooacetate 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.11 Where the coupling and hydrazone

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. 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 (21 → 27, Chart III). With potassium thiocyanate on 21, the 2-isothiocyanate compound 30 (Chart IV) was isolated instead of the expected 2-thiocyanato compound. 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.

When 32, the product from chloroacetyl chloride and 17, was allowed to react with thiourea, both chlorine atoms were displaced and a bisisothiouronium salt resulted (Chart V). Ordinarily, chloroacetyl groups are removed by thiourea in refluxing aqueous ethanol at pH 5 with the formation of pseudothiohydantoin. Therefore, we expected the 2-isothiourea derivative 34, but, under these conditions, isolated starting material 17. The pKₐ 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 indolene-2-thiols are inherently unstable. Likewise, 2-indolinols are known only as N-acyl or N-alkyl derivatives. 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)

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

\[ \text{Chart VI} \]

\[ \text{32} \xrightarrow{\text{Sb}^+} \text{35} \]

\[ \xrightarrow{\text{36}} \]

\[ \text{37, } n=1 \]

\[ \text{38, } n=2 \]

\[ \text{39, } n=4 \]

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.33

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 triphenylphosphine37 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 C7 proton (δ 7.70 and 8.20, respectively), indicative of increased deshielding by the carbonyl group in 38.

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

Accordingly, N-trifluoroacetylsericosine 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).

\[ \text{Chart VII} \]

\[ \]

As we have proposed elsewhere41 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 B1) or removal of the N-trifluoroacetyl group following reaction with polysulfide ions (pathway B2). 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 cyanoborohydride44 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 C8 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 C8 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)4–8 and sporidesmin (4)36 into monosulfides.


(42) The alkaline decomposition of organic disulfides very often produces monosulfides.44


The mixture of monosulfides 51 and 52 was heated with 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 R₁ value on tlc. 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).

The occurrence of two pairs of enantiomers could then be explained by epimerization at C₂' in 55. A deuteron-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 (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, making this mechanism unlikely.

Milder reaction temperatures and the use of non-protonic 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 (CHCl₃ or CCl₄) 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 (1993) described the synthesis of 11 and 2,2'-dibenzylandamino-2,2'-dithiodipropanoic acid, in which the amino function is acetylated. Interestingly, the decollected products were not mentioned.

(47) A Plattner rearrangement (1972) 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.
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with ether. The organic layer was washed with water, 5% NaOH (0.175 mol, 87% based upon recovered starting material) was stored

Dry Ice-acetone bath. After the solution was stirred for 45 min

Immediately after the addition of the sodium nitrite solution,

200 ml of ethanol was cooled at −15° (ice-salt bath). Just before

Solution A. A solution of 51.0 g (0.30 mol) of ethyl azolopyaconate (22) in 200 ml of ethanol was cooled at −15° (ice-salt bath). Just before

Solution B. A solution of 51.0 g (0.30 mol) of freshly distilled aniline, 255 ml

n

After addition of 5 ml of benzene, solvent and excess reagent were removed under vacuum distillation (34 mm) on a Vigreux column yielded two fractions, one spot; uv $\lambda_{max}$ $R_{f}$ 294 and 232 mm (equal intensities); ir (KBr) 3070, 2980, 2870, 1713 (CO), 1542, 1460, 1365, 1310, 1280, 1210, 1190, 1120, 1090, 1065, 1080, 860, 785, 770, and 750 cm$^{-1}$; $n$ 5.78 (mult, 1 H, C-H), 7.35 (mult, 3 H, C-C=C), 4.46 (q, 2 H, CH$_2$CH$_3$), 1.52 (s, 6 H, CH$_2$CH$_2$CH$_2$), 1.35 (t, 3 H, CH$_2$CH$_3$); mass spectrum (75°C, 70 eV) m/e 343 (M$^+$, 100%), 325 (M$^+$ - OC$_2$H$_4$), 143, 130, 128, 117, 115, 103, 91, 77.

The ester 17 was also prepared according to Robinson and Sugionome$^{11}$ from the hydrazine acid 25, yielding 6.5% of the ester 17 and 35.5% of the acid 26.

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


(q, 2 H, COOCH₃); 3.30 (q, 2 H, COOD); 3.31 (s, 2 H, -COCH₃); mass spectrum (180°), m/e 318 (M⁺), 302 (M⁺ - 60), 286 (M⁺ - 120), 270, 254, 238, 222, 206, 190, 174, 158, 142, 126, 110 (0.7 mm) (material that sublimed at a lower temperature was found to be starting material 37 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 sodium thioacetate (15 min). The reaction mixture was kept at 0° and stirred for 5 min, after which it was extracted twice with ice-cold ethyl acetate. The combined layers were dried (Na₂SO₄) and the solvent removed to yield 1.94 g of a dark brown oil; 0.65 g of this oil was subjected to preparative tlc on five plates (developed two times, 3% ethanol–benzene), to yield 192 mg (0.88 mmol, 38%) of starting material 270 and 270 mg of a still impure, ninhydrin-positive material. The latter was chromatographed 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 Rₜ 0.41. A small sample was crystallized from slowly evaporating chloroform: mp 101–103°; ir (CHCl₃) 2980, 1730 (ester), 1705 (amide), 1685 (amide C=O), 218, 217, 150 (base peak), 203, 172, 158, 145, 144.

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 reaction mixture was monitored by tlc. The solvent was removed and the residue subjected to preparative tlc (developed two times, 5% ethanol–benzene), to yield 9.5 mg (3.3 × 10⁻² mmol, 80%) of 37. Identical tlc was based upon ninhydrin and uv spectrometric analysis.

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

2-Carbethoxy-3,3-dimethylindolin-2-one (45). 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 solution of (0.33 mol) of powdered sulfur and 26 g (0.11 mol) of Na₂SO₄. Reaction conditions and work-up were the same as described above. After preparative tlc, 80 mg (27%) of monosulfide 37 and 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 thioacetate¹¹ (20 ml of a 1.1 M solution), respectively. Reaction conditions and work-up were as described above. Yield of disulfide bromosarcosine sulfate 110 (0.7 mm) (material that sublimed at a lower temperature was found to be starting material 37 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.

Analogs of Anhydrogliotoxin.

Within a few minutes a precipitate formed. The reaction mixture was kept at 0° and stirred for 15 min, after which the clear solution was refluxed for 15 min. The last aliquot was allowed to react with 20 ml of a 1.1 M aqueous solution of freshly prepared sodium thioacetate (15 min). The reaction mixture was kept at 0° and stirred for 10 min, after which it was extracted twice with ice-cold ethyl acetate. The combined organic layers were dried (Na₂SO₄) and the solvent removed to yield 1.94 g of a dark brown oil; 0.65 g of this oil was subjected to preparative tlc on five plates (developed two times, 4% ethanol–benzene) to yield 192 mg (0.88 mmol, 38%) of starting material 270 and 270 mg of a still impure, ninhydrin-positive material. The latter was chromatographed 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 Rₜ 0.41. A small sample was crystallized from slowly evaporating chloroform: mp 101–103°; ir (CHCl₃) 2980, 1730 (ester), 1705 (amide), 1685 (amide C=O), 218, 217, 150 (base peak), 203, 172, 158, 145, 144.

Monosulfide 37: ir (CHCl₃) 2960, 1730 (ester), 1705 (amide), 1685 (amide C=O), 218, 217, 150 (base peak), 203, 172, 158, 145, 144. Anal. Caled for C₃₁H₂₄N₂O₆S: C, 61.83; H, 5.88; N, 4.81; S, 10.76. Found: C, 61.5; H, 5.9; N, 4.8; S, 10.77.

Disulfide 38: ir (CHCl₃) 2970, 1732 (ester), 1505 (amidine), 1595, 1475, 1455, 1390, 1284, 1130, and 1025 cm⁻¹; νm 3.75 (mult, 1H, C=C); 7.18 (mult, 3H, C-H); 4.18, 4.2 H, CH₂CH₂); 4.07 (d, 1H, C=H, Jₐbd = 15 Hz, AB spectrum), 3.70 (d, 1H, C=H), 1.45 (s, 3H, C₃H₉); 1.35 (5, 3H, C₃H₉); 1.21, (t, 3H, C₃H₉); mass spectrum (180°), m/e 291 (M⁺), 218 (M⁺ – CO₂CH₃), 205, 183, 159, 145, 144.

Anal. Caled for C₃₃H₃₄N₂O₆S₂: C, 61.70; 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 as described above.

(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-λ)indole and 3,4,5,6,10a-hexahydro-4-keto-10,10-dimethyl-10a-carbethoxy-1,2,5-dithiazino(5,6-λ)indole, respectively. For convenience we use the names above.

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 \( R_t \). 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, \( R_t \) 0.50; ir (CHCl₃) 3400 (sharp, NH), 2980, 2940, 2850, 1730 (ester), 1640 (amide), 1600, 1525, 1480, 1375, and 1360 cm⁻¹; nmr \( \delta \) 8.15 (mult, 1 H, C₇–H), 7.20 (mult, 3 H, C₆–H), 5.50 (broad singlet, 1 H, C₁–H), 4.17 (q, 2 H, CH₂CH₃), 3.23 and 3.13 (2 singlets, separated 5 Hz, 3 H, N–CH₃), 2.0 (broad S, 1 H, NH), 1.47 and 1.29 (2 singlets, 6 H, CH₃CCH₃), 1.29 (t, 3 H, CH₃CH₃); mass spectrum (160°), \( m/e \) 320 (M⁺), identical with that for 51 and 52, except for a stronger signal at \( m/e \) 304 (M⁺ – CH₄) and a weaker one at \( m/e \) 247 (M⁺ – CO₂CH₂H₂) and 245.

The nmr spectrum of the isolated “starting material” showed a change in that the ratio of the two signals from the C₇-proton was reversed (now \( \delta 5.65/5.29 = 1:2 \)), indicating that only the cis enantiomers 51 have been isomerized.

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