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-polythiol mixture gave a monothiol 49 (30%) yield but no disulfide 50. Reduction of 49 with NaCNBH₃ proceeded stereoselectively to afford mainly the seco-gliotoxin 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 chaetomin (2) and verticillin A (3). Both are highly active against gram-positive bacteria. Chaetomin is cytotoxic but lacks antiviral activity, while verticillin A possesses potential antibacterial activity, the aranotins members of this group of fungal metabolites are the active against gram-positive bacteria. Chaetocin is chaetocin (2) and verticillin A (3). Both are highly active in inhibiting viral RNA synthesis, in support of the view 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. The drastic reaction conditions of all of these methods preclude their successful extension to the polycyclic epidithiodiketopiperazines. A synthetic approach of general applicability, we feel, would feature the initial construction of the disulfide bridge and then ring closure to a bridged diketopiperazine. The addition of acyl chlorides to indolines (Chart I), a reaction first reported by Leuchs, who studied compounds 14-16, served as our first step. The 2-chloro substituent in Leuchs' adducts 18-20 is known to undergo easy nucleophilic displacement, and known structure, probably contain the epidithiodiketopiperazine ring. The former is active against gram-positive bacteria and viruses while the latter has only antifungal and antiviral activity.

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

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

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(15) J. W. Moncrief, ibid., 90, 6517 (1968).
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-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\(^3\) 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 $\rightarrow$ 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 $\rightarrow$ 27, Chart III). With potassium thiocyanate on 21, the 2-isothiocyno compound 30 (Chart IV) was isolated instead of the expected 2-thioicyano 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 are removed by thiourea in refluxing aqueous ethanol at pH 5 with the formation of pseudothiohydantoin.\(^{(35)}\) 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 indolene-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)
and a disulfide (38) in yields varying with the reactant (Chart VI). Sodium sulfide and sodium thiocarbonate 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.

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 trimethylamine 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-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-trifluoroacetyl sarcosine chloride (42) was prepared from the free acid with thionyl chloride, conditions mild enough not to affect the trifluoroacetyl group. 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.

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).

As we have proposed elsewhere, 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-trifluoroacetylated group to yield 49 via 48 (pathway B1) or removal of the N-trifluoroacetylated 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.

The monosulfide 49 was reduced with sodium cyanoborohydride to the amines 51 and 52 (Chart VIII), which are secoglotoxin 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 C7 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 C7 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

Chart VI

![Chart VI](image)

Chart VII

![Chart VII](image)

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(37) This method has been used to convert dehydrogliotoxin (12b) and sporidesmin (4) into monosulfides.
(42) The alkaline decomposition of organic disulfides very often produces monosulfides.
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 \(\text{O} = \text{C} = \text{NCH}_3\) has little effect.

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\(^\circ\)C did a reaction occur yielding, besides starting material, a compound with a slightly higher \(R_1\) value on tlc.\(^{(46)}\) 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),

\[\text{Chart IX}\]

However, nmr spectra at \(-20\) or \(-40^\circ\) 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, \(\text{via}\) 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.\(^{(47,48)}\) 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 \(\alpha\)-thio-bridged \(\alpha\)-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 \(\alpha\)-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 \(\rightleftharpoons\) 55 + 56 showed only two spots on tlc with no trace of side products,\(^{(47)}\) making this mechanism unlikely.

\[\text{Chart VIII}\]

\[\text{Chart IX}\]

Milder reaction temperatures and the use of non-protic solvents provided no new information. At 90\(^\circ\) the formation of the new isomers is very slow and no new component could be detected; diglyme as solvent at 90 or 120\(^\circ\) 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\(_3\)) 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 \(\delta\) 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'-dibenzamidino-2,2'-dithiodipropanoic acid, in which the amino function is acylated. Interestingly, the decacylated products were not mentioned.

\((47)\) A Plancher rearrangement\(^{(27)}\) 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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otherwise. Melting points were taken on a Kofler hot stage and are corrected. Thin layer chromatography (tlc) was carried out using Merck precoated silica 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 (28). This compound was prepared from ethyl acetoacetate and 2-bromopropane following the procedure for the synthesis of ethyl n-propylacetoacetate. 1 Vac- 

duum distillation (34 mm) on a Vigoex column yielded two fractions, bp 83–105° and 105–108°. The addition product of 434 mg (2 mmol) of the crude hydrazone ester 16 with 25 ml of concentrated aqueous NH4Cl, this oil was extracted with ether and the ether was removed to yield 545 mg of a yellow oil: tlc (4% ethanol-benzene), one spot was observed in tlc, which had the same Rf value as the starting material: ir (CHCl3) 2980 (broad), 2040 (strong, broad, —N = C = S 33), and 1700 cm–1; mass spectrum (190°) m/e 266 (190°), 251 (190° - 15), 234 (M + ), 219 (M + - CH3), 199, 191 (M + - CO2CH3), 188, 173, 172 (M + - CH3CON), 160 (M + - CH3CON - CO), 145 (160 - CH3), 105, 92 (alilicene – I).

α-Keto-β-methylbutyric acid phenylhydrazone (25). This compound was prepared12 in 20% yield from the starting material: ir (CHCl3) 1750, 1690 cm–1, disappearance of the starting material; uv (CHCl3) 248 nm, shoulders at 278 and 286 nm (specific rotations assigned to the free amine).

3-Dimethylindolene-2-carboxylate (17). An ice-cooled solution of 12.0 g (51 mmol) of the crude hydrazone ester 16 in 20% yield and showed on tlc (3% acetic acid–benzene) only one spot: uv XmaxEtOH 341 nm, shoulders at 215 and 330 nm. The dark red residue (48.0 g, 0.163 mol, 96%) was dissolved in 50 ml of water, cooled at –15°, an oil separated from the dark red colored reaction solution B was added with swirling to solution A and cooled in a Dry ice–acetone bath. After the addition of solution B to A, 232.5 ml of 5 M NaOH (1.15 mol), dissolved in 50 ml of water. All solutions were cooled at –15°. The organic layer was washed with water, 5% NaHCO3, and filtered, this oil was extracted with ether, and finally water until neutral, dried (Na2SO4), and filtered, and the ether was removed to yield 38.5 g (0.163 mol, 96%), and the ether was removed to yield 38.5 g (0.163 mol, 96%), and the ether was removed to yield 38.5 g (0.163 mol, 96%)

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(q, 2 H, COCHCH3), 3.30 (q, 2 H, COCHCH3), 2.35 (3 H, CH3CON), 1.45 (6, 6 H, CH2C6H4), 1.32 (7 H, -COOC6H5CH3), 1.13 (7 H, 3 H, COCHCH3); mass spectrum (160) m/e 305 (M+), 275 (M+ - CH4), 232 (275 - CH3CO), 218, 217, etc.

2.9-Bisathiouroum-1-acyetyl-2-carbethoxy-3,3-dimethylindoline Di-C-closphate (33). The addition product of 17 (434 mg, 2 mmol) and 3.3 g (26 mmol) of powdered sulfur in 25 ml of benzene was refluxed for 5 min, then the volume was reduced to 5 ml, and from the filtrate the solvent removed to yield 395 g of a yellow powder: uv \( \lambda_{max} \) 210 (773, H2O) cm-1; nmr \( \delta \) 7.17, 7.14 (1 H, C H-C), 7.18 (2 H, C H2), 2.05 (3 H, CH3); mass spectrum (160) m/e 274 (M+), 252, 235, 218, 217, etc.

Conversion of 38 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 cm-1; nmr cold solution of sodium disulfide (1.12 (M+ - S2), 252, 250 (M+ - COONa), 232 (250 - CH3CO), 218, 217, etc.

2,3-Dimethylindoline-2-carbethoxy-3,3-dimethylindoline Di-C-chloride (33). The addition product of 17 (434 mg, 2 mmol) and 3.3 g (26 mmol) of powdered sulfur in 25 ml of benzene was refluxed for 5 min, then the volume was reduced to 5 ml, and from the filtrate the solvent removed to yield 395 g of a yellow powder: uv \( \lambda_{max} \) 210 (773, H2O) cm-1; nmr \( \delta \) 7.17, 7.14 (1 H, C H-C), 7.18 (2 H, C H2), 2.05 (3 H, CH3); mass spectrum (160) m/e 274 (M+), 252, 250 (M+ - COONa), 232 (250 - CH3CO), 218, 217, etc.

Conversion of 38 into Monosulfolide 37. A solution of 13 mg (4.1 × 10-2 mmol) of 38 and 22 mg (8.4 × 10-4 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 tic. The solvent was removed and the residue subjected to preparative tic (developed two times, 3% ethanol-benzene), to yield 9.5 mg (3.5 × 10-2 mmol, 80%) of 37. Identical values, uv, and ir spectra identical with those of 38.

Conversion of 38 into Monosulfolide 37. A solution of 13 mg (4.1 × 10-2 mmol) of 38 and 22 mg (8.4 × 10-4 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 tic. The solvent was removed and the residue subjected to preparative tic (developed two times, 3% ethanol-benzene), to yield 9.5 mg (3.5 × 10-2 mmol, 80%) of 37. Identical values, uv, and ir spectra identical with those of 38.

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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 Rf. 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, Rf 0.50; ir (CHCl₃) 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, Cr-H), 7.20 (mult, 3 H, C₅–H), 5.50 (broad singlet, 1 H, C₂–H), 4.17 (q, 2 H, CH₃CH₃), 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⁺ − CO₂C₇H₄) 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 δ 5.65/5.29 = 1:2), indicating that only the cis enantiomers 51 have been isomerized.

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