The Synthesis of Cyclopenin and Isocyclopenin[1]

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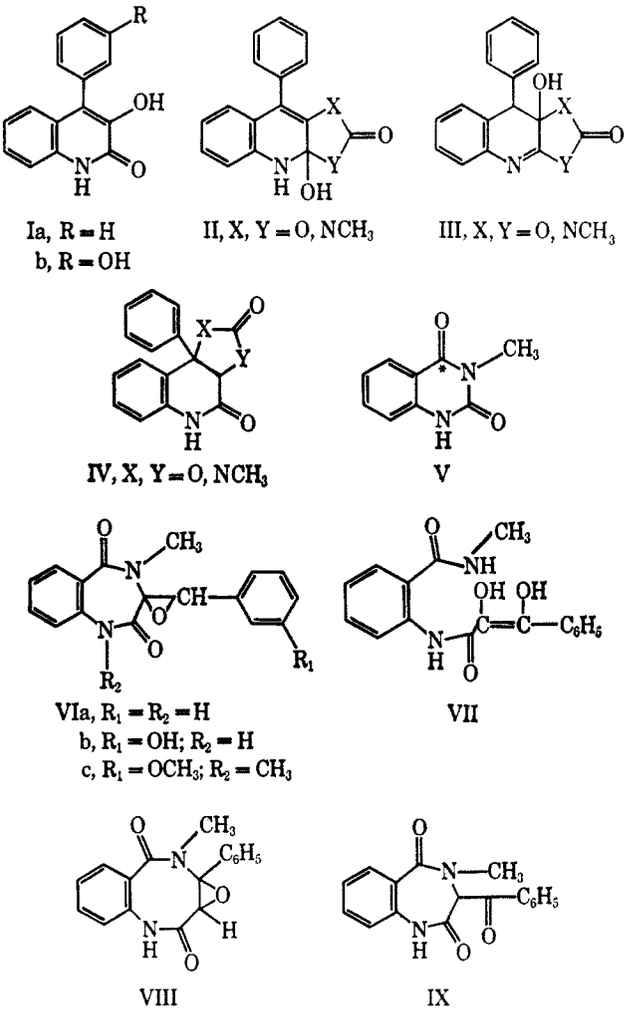
The spiro(styrene oxide-3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione) structure postulated for cyclopenin, a metabolite of *Penicillium cyclopium*, has been confirmed by synthesis. The synthesis proceeded *via* condensation of 3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione with benzaldehyde to give a mixture of the 3-*cis*- and *trans*-benzylidene compounds which were separated by chromatography and stereochemically characterized by nuclear magnetic resonance. Epoxidation of the *trans* isomer with *m*-chloroperbenzoic acid led to *dl*-cyclopenin whereas *dl*-isocyclopenin was obtained by similar epoxidation of the *cis* isomer. Both cyclopenin and isocyclopenin give viridicatin on treatment with hydrochloric acid.

Penicillium cyclopium and related mould species are the source of a number of nitrogeneous metabolites, including cyclopenin, cyclopenol, viridicatin, and viridicatol. The structures of viridicatin and viridicatol have been established as 3-hydroxy-4-phenyl-2-quinolone and 3-hydroxy-4-(3-hydroxyphenyl)-2-quinolone (Ia and Ib), respectively, by degradation and by synthesis.[4-7] Cyclopenin, $C_{17}H_{14}N_2O_3$, first isolated[5] from *P. cyclopium* Westling, was subsequently shown[6] to be accompanied by and separable from a closely related phenolic metabolite, cyclopenol, $C_{17}H_{14}N_2O_4$.

Cyclopenin and cyclopenol are optically active and in dilute acid or alkali their optical activity is lost with concomitant appearance in high yield of viridicatin (Ia)[5] and viridicatol (Ib),[6] respectively, and methylamine and carbon dioxide. To accommodate these observations, a quinolinooxazolidinone structure (II or III) was assigned[5] to cyclopenin, and it was subsequently pointed out[8] that fusion at the 3,4 position (IV) was also permissible.

An extensive reinvestigation[7] of the metabolites of *P. cyclopium* and *P. viridicatum* disclosed a number of features incompatible with the quinolinooxazolidinone structures. Cyclopenin-^{14}C, prepared by *in vivo* incorporation of anthranilic acid-^{14}COOH, was degraded with loss of carbon dioxide-^{14}C to radioinactive viridicatin by dilute acid. An enzyme preparation from the mycelium of *P. viridicatum* also converted cyclopenin into viridicatin with carbon dioxide evolution.[7] Biosynthetic studies further indicated that the intact carbon skeleton of phenylalanine serves as precursor for viridicatin and viridicatol[9,10a] and for cyclopenin and cyclopenol.[10b] Incorporation of anthranilic acid and phenylalanine into cyclopenin accounted for all of the carbons except the N-methyl carbon, which presumably was derived from methionine.[7]

More cogent evidence for dismissing structures II, III, and IV was the observation that oxidation of cyclopenin with hydrogen peroxide in acetic acid yielded



3-methyl-2,4-quinazolinedione (V),[7] benzoic acid, benzaldehyde, carbon dioxide, and anthranilic acid, radioactive quinazoline V being obtained from radioactive cyclopenin. On the basis of these data Luckner and Mohammed[7] proposed that cyclopenin (VIa) and cyclopenol (VIb) are seven-membered cyclic dipeptides formed from anthranilic acid and phenylalanine with an N-methyl group supplied by methionine. The epoxide moiety of VIa was suggested as more consistent with the ir absorption than hydroxyl or ketone groups. Further chemical and spectroscopic support cited[4] for the proposed structure of cyclopenin were (a) infrared (ir) absorptions at 3900 (NH), 1700, 1630 (CON), 990, and 885 cm^{-1}; (b) nuclear magnetic resonance (nmr) singlet at δ 4.04 (benzylic epoxide proton); and (c) reaction with diazomethane in which cyclopenin and

(1) (a) Supported in part by the U. S. Army Research Office, Durham, N. C. (b) Part of these results have been reported in a preliminary communication [H. Smith, P. Wegfahrt, and H. Rapoport, *J. Amer. Chem. Soc.*, **90**, 1668 (1968)].

(2) U. S. Public Health Service Postdoctoral Fellow.

(3) National Institutes of Health Predoctoral Fellow.

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(5) A. Bracken, A. Pocker, and H. Raistrick, *ibid.*, **57**, 587 (1954).

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(7) M. Luckner and Y. S. Mohammed, *Tetrahedron Lett.*, 1987 (1964).

(8) J. T. Edwards, *Ann. Rept. Prog. Chem.* (Chem. Soc., London), **51**, 247 (1954).

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(10) (a) Y. S. Mohammed and M. Luckner, *Tetrahedron Lett.*, 1953 (1963); (b) M. Luckner, *European J. Biochem.*, **2**, 74 (1967).

cyclopenol form mono- and dimethyl derivatives, respectively.

The above spectroscopic, chemical, and biosynthetic data, while providing strong support, do not unambiguously delineate the structure of cyclopenin. First, the facile acid or alkaline decarboxylation and rearrangement of cyclopenin to viridicatin is not consistent with known decarboxylation rates of anthranilic acid and its derivatives.[11] Opening of the epoxide to yield the suggested[7] intermediate VII followed by quantitative liberation of methylamine and carbon dioxide from the acylaminobenzamide derivative with ring closure to viridicatin seems unlikely under the mild conditions employed.

Second, O-methyl analysis of methylcyclopenin and dimethylcyclopenol indicated none in the former and one O-methyl group in the latter, requiring that the other methyl be located on nitrogen or carbon. The ir assignments[10] of the 1680- and 1655-cm^{-1} bands to the CONH group in methylcyclopenin and the 1690- and 1645-cm^{-1} bands to the CONH group in dimethylcyclopenol imply that the other methyl group is on carbon, a conclusion in conflict with structure VI.

Third, the reported spectroscopic properties of cyclopenin are inadequate to differentiate VIa from related structures, *e.g.*, VIII and IX, which could accommodate the ir and nmr data. Therefore, our first objective was to prepare model compounds for spectral correlation with cyclopenin and this was closely followed by efforts to synthesize the postulated structure VIa.

Results and Discussion

Cyclopenin and cyclopenol were obtained[12] from the culture filtrate of *P. cyclopium* Westling by a modification of the described procedure.[5] Since the styrene oxide portion should have little effect on the characteristic ultraviolet (uv) absorption of cyclopenin [λ_{max} 211 nm (ϵ 37,200), 290 (2060)], a reasonable model would be 3,4-dihydro-1H-1,4-benzodiazepine-2,5-dione (Xa).[13] The similarity of the spectra of Xa [λ_{max} 215 nm (ϵ 41,200), 272 (12,500), 293 (3500)] and cyclopenin prompted synthesis of 3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione (Xb)[14] which was accomplished *via* ethyl *o*-nitrobenzoylsarcosinate without isolation of the intermediate amine. The uv absorption of Xb [λ_{max} 215 nm (ϵ 32,100), 291 (2180)] is in full correspondence with that of cyclopenin.

To help to settle the question of the diazomethane reaction with cyclopenin and cyclopenol, 3,4-dihydro-1,4-dimethyl-1H-1,4-benzodiazepine-2,5-dione (Xc) was prepared by methylation of Xb with diazomethane or by the procedure of Lee.[15] The spectrum of Xc [λ_{max} 288 nm (ϵ 1970)] is quite similar to that of methylcyclopenin and dimethylcyclopenol. This indicates that diazomethane reaction has indeed led to N methylation and this was confirmed by repeating the methylation of cyclopenol with diazomethane. Dimethylcyclopenol has three-proton singlets at δ 3.69, 3.43, and 3.72. Since it has been established that dimethylcyclopenol contains one O-methyl group, the other new methyl group must be N-methyl in accordance with its chemical shift, and the original ir assignments[10] are incorrect. Thus cyclopenin and cyclopenol contain an acidic NH group, as does the model benzodiazepine Xb.

Although no analogy could be found for the extremely facile loss of carbon dioxide from cyclopenin upon treatment with acid (*e.g.*, the benzodiazepines X lost less than 10 mol % carbon dioxide upon boiling in 2 *N* hydrochloric acid for 3 hr), the chemical and spectroscopic evidence now seemed sufficiently persuasive to accept structure VIa as an objective for synthesis. However, before undertaking its synthesis, degradation of cyclopenin to a simpler compound containing some of its unique features was undertaken.

The route chosen was to deepoxidize cyclopenin and then to synthesize the corresponding olefin. For example, ethyl phenylglycidate has been converted into ethyl cinnamate on treatment with thiourea[16] or tributylphosphorus.[17] Also, chalcone oxide[18] with chromous chloride in acetic acid was rapidly deepoxidized. Cyclopenin was unchanged on treatment with chromous chloride. Although cyclopenin reacted slowly with tributylphosphorus at 85° during 48 hr, most was recovered; phenylglycidanilide afforded cinnamanilide under these conditions. Phenylglycidanilide also yielded cinnamanilide in excellent yield after 4-hr reaction with thiourea. Under similar conditions with thiourea cyclopenin was partially converted after 7 days reaction into a product, tentatively assigned structure XIa or XIb.[19] Attempts to convert this thiourea derivative into deoxycyclopenin were not successful. The sluggish reactions with thiourea and thiosulfate,[20] respectively, indicate then the epoxide group of cyclopenin is in an unusual and unreactive environment. Failure in these degradations attempts turned our efforts directly to synthesis.

The synthesis of the isomeric *dl*-cyclopenins of structures XIIa and XIIb required a 3-substituted benzodiazepine-2,5-dione, and, among the numerous 1,4-benzodiazepine and 1,4-benzodiazepinedione derivatives,[21,22] few with 3 substituents have been described.[13,23] Our approach was to synthesize first the benzylidene compounds XIc and XId *via* Xb in analogy to similar condensation of amides, hydantoin, diketopiperazine, and creatine with benzaldehyde.[24-26] Condensation of Xb with benzaldehyde using Perkin reaction conditions[27] gave the isomeric 3-benzylidene-4-methyl-1H-1,4-benzodiazepine-2,5-diones (XIc and XId) and the N-acetyl derivative XIe. An alternative

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(12) The crude isolate was obtained by H. R. while a guest in the laboratory of Dr. H. Raistrick during March 1956.

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(14) While this work was in progress the synthesis of Xb was reported by P. M. Carabateas and L. S. Harris, *J. Med. Chem.*, **9**, 6 (1966).

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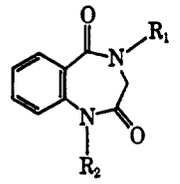
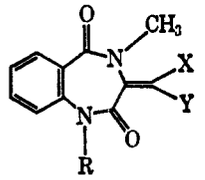
synthesis of XIc and XId *via* *o*-nitrobenzoylsarcosine and condensation with benzaldehyde to XIVa followed by reduction and ring closure gave an inferior yield. Also, Xc was unreactive under conditions which gave the monomethylbenzylidine derivative XIc.

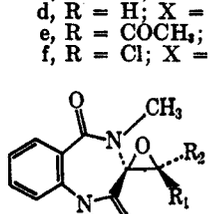
That XIc and XId have the stereochemistry shown is evident from examination of their respective nmr spectra. The major product, mp 207–208°, assigned structure XIc, showed N-methyl and vinyl hydrogen resonances as singlets at δ 3.20 and 6.95, respectively. The minor isomeric product, mp 185°, exhibited N-methyl resonance at δ 3.50 and vinyl hydrogen resonance at 6.72. The upfield shift (18 Hz) observed for the N-methyl resonance of the major isomer (XIc), with respect to that of the minor isomer (XId), is consonant[28] with its interaction with the π electrons of the proximate benzene ring. This interaction requires a *cis* stereochemistry for the two groups. The expected[25,28] downfield shift (13.8 Hz) of the vinylic hydrogen of XIc relative to XId caused by interaction with the 2-carbonyl was also observed.



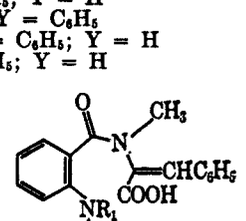
Xa, $R_1 = R_2 = H$
b, $R_1 = CH_3$; $R_2 = CH_3$
c, $R_1 = R_2 = CH_3$



XIa, R = H; X = C_6H_5; Y = $SC(=NH)NH_2$
b, R = H; X = $SC(=NH)NH_2$; Y = C_6H_5
c, R = H; X = C_6H_5; Y = H
d, R = H; X = H; Y = C_6H_5
e, R = $COCH_3$; X = C_6H_5; Y = H
f, R = Cl; X = C_6H_5; Y = H



XIIa, $R_1 = H$; $R_2 = C_6H_5$
b, $R_1 = C_6H_5$; $R_2 = H$



XIIIa, $R_1, R_2 = O_2$
b, $R_1 = H$; $R_2 = COCH_3$

Alkaline hydrolysis of the N-acetyl compound XIe resulted in preferential cleavage of the benzodiazepine ring to yield XIIIb. However, in view of the ketonic character of imides, sodium hypochlorite was next tried and the N-acetyl group was successfully removed, yielding the N-chloro derivative XIf. This was readily decomposed in ethanol or with potassium iodide and thiosulfate to give XIc.

Epoxidation of XIc was complicated by adverse steric and electronic features, and no precedent exists for epoxidation of a double bond so substituted. Numerous attempts were made, without success, to epoxidize XIc with alkaline oxidizing agents[29] and trifluoroperacetic acid;[30] all gave complex mixtures of products. Prolonged reaction of XIc with *m*-chloroperbenzoic acid gave a product in 37% yield, $C_{17}H_{14}N_2O_3$, mp 194–195°, which was readily hydrolyzed to viridicatin (I) in 2 *N* hydrochloric acid solution. The ir and uv spectra (Table I) of the product are identical with those of *l*-cyclopenin; the nmr spectrum of the epoxide shows the same aromatic hydrogens multiplet at δ 6.6–7.8 as cyclopenin, and the N-methyl and benzylic hydrogens have the same chemical shift (δ 3.20 and 3.94, respectively) and the same ratio (3:1) as those observed for natural cyclopenin. Further, the anomalous nmr singlets of cyclopenin at δ 4.51 and 2.55 were observed in the synthetic product.[31] Finally, the mass spectra of *dl*-cyclopenin and *l*-cyclopenin were superimposable. Cyclopenin is therefore assigned structure XIIa as required from the *cis* relationship of the N-methyl and phenyl groups of the immediate precursor XIc.

The product obtained by oxidation of XId, $C_{17}H_{14}N_2O_3$, was easily converted into viridicatin; its uv spectrum exhibited maxima at 290 and 211 nm; and its ir spectrum exhibited carbonyl absorptions at 5.85, 6.05 and 7.2 μ. The nmr spectrum (Table I) showed proton resonances at δ 6.4–7.8, 3.82, and 3.11, in a ratio of 9:1:3 and were assigned to aromatic, benzylic, and N-methyl hydrogens, respectively; this product therefore is isocyclopenin (XIIb).

Experimental Section[32]

Cyclopenin (VIa) and Cyclopenol (VIb).—Cultures of *P. cyclopium* LSHTM strain no. 72 were harvested after 15 days. The culture was filtered, the mycelium was pressed, the combined filtrates were adjusted to pH 2 with concentrated hydrochloric acid, and the solution was treated with 120 g of charcoal. The mixture was stirred for 2 hr and filtered and the charcoal was washed with five 500-ml volumes of water. Chloride ion was not detected after the third wash. The charcoal was dried *in vacuo*, slurried with 1 l. of methanol, and packed into a column, eluting with four 2-l. portions of methanol. Evaporation of the first two portions and crystallization of the residue from methanol gave 3.5 g as a mixture of cyclopenin and cyclopenol. Fractional crystallization of the mixture from ethyl acetate–benzene (1:3 v/v) afforded impure cyclopenol. Cyclopenin was obtained from the mother liquors by (a) paper chromatography,[6] (b) silica gel chromatography (elution with 3:1 methylene chloride–benzene), or (c) alumina chromatography (elution with 3:1 benzene–ethyl acetate); the last procedure gave a poor recovery of cyclopenol.

Recrystallization of chromatographed cyclopenin from ether–methylene chloride solution gave pure cyclopenin (VIa): mp 179–180°; $[\alpha]_{5461}^{23}$ −301° (*c* 1.0, methanol) [lit.[6] mp 183–184°; $[\alpha]_{5461}^{20}$ −291° (*c* 1.0, methanol)].

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(29) (a) W. C. Anthony, *J. Org. Chem.*, **31**, 77 (1966); (b) G. B. Payne, *ibid.*, **23**, 2048 (1958); (c) E. C. Kornfeld, E. J. Kornfeld, G. B. Kline, M. J. Mann, and D. E. Morrison, *J. Amer. Chem. Soc.*, **78**, 3087 (1958); (d) N. C. Yang and F. A. Finnegan, *ibid.*, **80**, 5848 (1958).

(30) W. D. Emmons and A. S. Pagano, *ibid.*, **77**, 89 (1955).

(31) These anomalous minor peaks are tentatively suggested to result from conformational equilibria and will be considered in detail in a future paper.

(32) Nmr spectra were determined with Varian A-60 and HA-100 instruments, using tetramethylsilane as internal reference (δ 0). Melting points, uncorrected, were determined on a Mel-Temp melting point apparatus. Ir spectra were determined for Nujol suspensions with Perkin-Elmer Model 127 and Model 137 instruments unless otherwise noted. Uv spectra were obtained with a Cary Model 14 spectrophotometer. Mass spectra were obtained with a Varian M-66 spectrometer; microanalyses were performed by the Micro Analytical Laboratories, University of California at Berkeley, Berkeley, Calif.

TABLE I

SPECTROPHOTOMETRIC PROPERTIES OF CYCLOPENIN AND SOME RELATED COMPOUNDS

Compound	Mp, °C	Uv, nm (ε)[a]	Nmr, δ[b]	Ir, μ[c]
Xa	327–327.5	293 (3,500) 272 (12,500) 215 (41,250)		3.07 (NH), 5.85, 5.93 (CONH)
Xb	241–242	291 (2,180) 215 (32,140)	7–7.9 (m, 4, ArH), 3.38 (s, 2, CH_2), 3.11 (s, 3, NCH_3) (in d_6-DMSO)	5.89 (CONH), 6.10 ($CONCH_3$)
Xc	142–143	288 (1,970)	7.2–8.2 (m, 4, ArH), 4.05 (q, 2, CH_2), 3.4 (s, 3, NCH_3), 3.4 (s, 3, NCH_3), 3.52 (s, 3, NCH_3)	5.97, 6.09 ($CONCH_3$)
XIc	207–208	286 (12,400) 255 (14,200) 239 (15,800) 213 (37,400)	7.38 (m, 9, ArH), 6.95 (s, 1, = CH), 3.20 (s, 3, NCH_3)	3.05 (NH), 5.90, 6.10 (CON), 6.20
XId	185	276 (15,600) 245 (18,300) 215 (43,700)	7.27 (m, 9, ArH), 6.72 (s, 1, =CH), 3.50 (s, 3, NCH_3)	5.93, 6.15 (CON)
l XIIa	179–180	290 (2,060) 211 (37,200)	7.30, 7.10, 6.56 (m, 9, ArH), 3.95 (s, 1, CHAr), 3.20 (s, 3, NCH_3) [4.51 (s, 0.08, CHAr), 2.56 (s, 0.24, NCH_3)]	2.97, 3.11 (NH), 5.85 (CONH), 6.0 ($CONCH_3$), 7.22, 9.12
dl XIIa	193–195	290 (2,100) 211 (37,900)	7.30, 7.10, 6.56 (m, 9, ArH), 3.94 (s, 1, CHAr), 3.20 (s, 3, NCH_3) [4.51 (s, 0.08, CHAr), 2.56 (s, 0.24, NCH_3)]	2.97, 3.11 (NH), 5.84 (CONH), 6.05 ($CONCH_3$), 7.22, 9.12[d]
XIIb	210–213	290 (2,200) 212 (34,200)	7.85, 7.12, 6.46 (m, 9, ArH), 7.12, 6.46 (m, 9, ArH), 3.82 (s, 1, CHAr), 3.14 (s, 3, NCH_3)	2.95 (NH), 5.85 (CONH), 6.05 ($CONCH_3$), 7.20
VIc	168–168.5	284 (3,340)	6.8–7.7, 6.12 (m, 8, ArH), 3.85 (s, 1, CH), 3.69 (s, NCH_3), 3.43 (s, 3, NCH_3), 3.72 (s, 3, OCH_3)	

[a] In ethanol. [b] In $CDCl_3$ with internal tetramethylsilane (TMS) unless otherwise stated: s, singlet; m, multiplet; q, quartet. [c] In Nujol except where indicated. [d] In chloroform.

Anal. Calcd for $C_{17}H_{14}N_2O_5$: C, 69.4; H, 4.8; N, 9.5; mol wt, 294. Found: C, 69.2; H, 4.9; N, 9.3; mol wt, 294 (mass spectroscopy).

Cyclopenol (VIb) was recrystallized from ethyl acetate–benzene: mp 210–211°; $[\alpha]^{20}_{5462}$ −310° (*c* 1.0, methanol) [lit.[6] mp 215°; $[\alpha]^{20}_{5461}$ −309° (*c* 1.3, methanol)]; uv λ_{max} 285 nm (ε 3740); nmr (CD_3OD) δ 3.11 (s, 3, NCH_3), 3.94 (s, 1, CHAr), 6.07–7.8 (m, 8, ArH).

Anal. Calcd for $C_{17}H_{14}N_2O_4$: C, 65.8; H, 4.6; N, 9.0. Found: C, 66.1; H, 4.8; N, 9.2.

Dimethylcyclopenol (VIc).—A solution of 200 mg of cyclopenol (VIb) in cold methanol was treated with a 4 *M* excess of ethereal diazomethane for 18 hr. The solution was filtered, evaporated to dryness, and chromatographed on silica gel to yield 62 mg of dimethylcyclopenol: mp 168–168.5° (lit.[6] mp 167–169°).

Anal. Calcd for $C_{19}H_{18}N_2O_4$: C, 67.4; H, 5.4; N, 8.3; mol wt, 338. Found: C, 67.4; H, 5.2; N, 8.1; mol wt, 338 (mass spectroscopy).

Ethyl o-Nitrobenzoylsarcosinate.—A suspension of 15.35 g (0.1 mol) of ethyl sarcosinate hydrochloride[33] in 150 ml of chloroform was stirred vigorously at 0° while 18.5 g (0.1 mol) of *o*-nitrobenzoyl chloride[34] in 20 ml of chloroform was added slowly. Stirring was continued for 21 hr at room temperature followed by successive washings with water, 0.1 *N* hydrochloric acid, 0.01 *N* sodium hydroxide, and water. Drying and removal of solvent gave a residue which was short path distilled at 110° (50 μ) to afford 26.6 g (75% yield) of ethyl *o*-nitrobenzoylsarcosinate.

Anal. Calcd for $C_{12}H_{14}N_2O_5$: C, 54.1; H, 5.3; N, 10.6. Found: C, 53.7; H, 5.1; N, 10.7.

3,4-Dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione (Xb).—A solution of 15 g (56 mmol) of ethyl *o*-nitrobenzoylsarcosinate (XIa) in 120 ml of ethanol containing 600 mg of platinum oxide was hydrogenated at 40 psi for 45 min. The residue from filtration and evaporation of the mixture was recrystallized from methanol to give 6.7 g (63% yield) of Xb: mp 241–243° (lit.[18] mp 246–247°), uv λ_{max} 215 nm (ε 32,140), 291 (2180); ir (Nujol) 5.88 (CONH), 6.10 μ ($CONCH_3$); nmr (d_6-DMSO) δ 7–7.9 (m, 4, ArH), 3.83 (s, 2, CH_2), 3.11 (s, 3, NCH_3).

Anal. Calcd for $C_{10}H_{10}N_2O_2$: C, 63.1; H, 5.3; N, 14.7; mol wt, 190. Found: C, 63.0; H, 5.2; N, 14.5; mol wt, 190 (mass spectroscopy).

3,4-Dihydro-1,4-dimethyl-1H-1,4-benzodiazepine-2,5-dione (Xc).—A solution of 190 mg (1 mmol) of Xb in 30 ml of methanol was treated with 50 ml of ethereal diazomethane (5 mmol) and the solution was stored at 0° overnight. Evaporation of solvent gave 140 mg of product which contained some Xb. Fractional recrystallization from an ethanol–water solution gave 40 mg of Xc: mp 142–143° (lit.[15] mp 146–147°); uv λ_{max} 288 nm (ε 1970); nmr δ 3.4 (s, 3, NCH_3), 3.52 (s, 3, NCH_3).

Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.7; H, 5.9; N, 13.7; mol wt, 204. Found: C, 64.8; H, 5.9; N, 13.7; mol wt, 204 (mass spectroscopy).

Decarboxylation of 3,4-Dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione (Xb).—A suspension of 380 mg (2 mmol) of Xb in 30 ml of 2 *N* hydrochloric acid was boiled in a stream of carbon dioxide free nitrogen. The effluent gas was passed through two bubblers containing carbon dioxide free 0.1 *N* sodium hydroxide for 3 hr. Addition of saturated barium chloride solution to the bubblers gave a precipitate of barium carbonate (40 mg) indicating that ~10 mol % of the theoretical amount of carbon dioxide had been evolved. The hydrolysis solution was evaporated to dryness and a small portion of the residue was examined by paper chromatography. Sarcosine, *o*-aminobenzoylsarcosine, and *o*-methylaminoacetylaminobenzoic acid were identified by comparison with authentic samples using 1-butanol–acetic acid–water (4:1:5) as the chromatographic solvent.

1-Acetyl-3-*trans*-benzylidine-3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione (XIe).[35]—A mixture of 2.66 g (14 mmol) of Xb, 2.2 g (10.8 mmol) of benzaldehyde, and 125 g

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(35) *cis* and *trans* designations are based on the cinnamate portion of the molecule.

(15.2 mmol) of sodium acetate in 4 ml of acetic anhydride was heated with stirring at 140° for 2 hr. Water was added, the solution was stirred, and the aqueous phase was decanted. The oily residue was trituated with ether and unreacted Xb was removed by filtration. The filtrate was evaporated. The residue was dissolved in chloroform, washed successively with sodium bisulfite and water, dried, and evaporated to a red viscous liquid which deposited 0.9 g of XIe: mp 177–179° from methanol solution; uv λ_{max} 245 nm (ϵ 14,500), 283 (13,320); nmr ($CDCl_3$) δ 7.5 (m, 9, ArH), 7.16 (s, 1, =CH), 3.3 (s, 3, NCH_3), 2.68 (s, 3, $NCOCH_3$).

Anal. Calcd for $C_{19}H_{16}N_2O_3$: C, 71.2; H, 5.0; N, 8.7. Found: C, 71.0; H, 5.0; N, 8.7.

3-*trans*-Benzylidine-1-chloro-3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione (XIf).—A solution of 0.192 g (0.6 mmol) of N-acetyl compound XIe in 2 ml of dioxane and 1 ml of 5.25% sodium hydrochlorite solution was stirred at room temperature for 5 min. The solution was diluted with water and extracted with chloroform and the combined extracts were washed with water, dried, and evaporated to a residue. The residue was recrystallized from chloroform–petroleum ether to give 0.131 g of XIf: mp 129–130°; uv λ_{max} 213 nm (ϵ 40,000), 243 (16,300), 283 (13,570).

Anal. Calcd for $C_{17}H_{13}N_2O_2Cl$: C, 65.3; H, 4.2; N, 9.0. Found: C, 65.2; H, 4.3; N, 9.0.

3-*trans*-Benzylidine-3,4-dihydro-4-methyl-1H-1,4-benzodiazepine-2,5-dione (XIc) from XIf.—Potassium iodide in 1 ml of 3% aqueous acetic acid was added in portions to a solution of 0.131 g of XIf in 1 ml of dioxane followed by decolorization with 0.1 *M* sodium thiosulfate solution. The reaction mixture was extracted with chloroform. The extract was dried and evaporated to a semicrystalline residue, which was recrystallized from chloroform–petroleum ether solution to yield 0.11 g of XIc: mp 201–202°.

3-*cis*- and -*trans*-Benzylidine-3,4-dihydro-4-methyl-1H-4-benzodiazepine-2,5-diones (XIc and XId).—A solution of Xb (5.32 g) in 8 ml of acetic anhydride was treated with 2.5 g of fused sodium acetate and 4.4 g of benzaldehyde. The mixture was heated at gentle reflux temperature for 2 hr and evaporated to a semisolid mass, and the residue was repeatedly concentrated from toluene, then suspended in water. The oily precipitate was extracted into methylene chloride, washed with 5% sodium bicarbonate, dried, and evaporated and the residue was placed onto a solumn of 60 g of activity III Woelm alumina. Elution with benzene gave 0.87 g of *trans* XIc, mp 201–205°, and further elution with benzene–methylene chloride (3:1) gave *cis* XId, mp 185°.

Anal. Calcd for $C_{17}H_{14}N_2O_2$: C, 73.4; H, 5.1; N, 10.1. Found for XIc: C, 73.0; H, 5.4; N, 10.2. Found for XId: C, 73.1; H, 5.0; N, 10.1.

α-(N-Methyl-N-*o*-nitrobenzoyl)aminocinnamic Acid (XIIIa).—A mixture of 3.33 g (14 mmol) of *o*-nitrobenzoylsarcosine,[36] 2.2 g (20.8 mmol) of benzaldehyde, and 1.25 g (15.2 mmol) of sodium acetate in 4 ml of acetic anhydride was heated for 4 hr. Ice–water was added to the stirred solution, the aqueous layer was decanted, and the residual oil was dissolved in 5% sodium bicarbonate solution and washed with methylene chloride. The aqueous layer was acidified to pH 2 and extracted with chloroform. The extract was dried and evaporated and the residue was recrystallized from methanol–petroleum ether to yield 3.96 g of XIIIa: mp 182–183°; uv λ_{max} 264 nm (ϵ 20,100); nmr δ 7.3–8.5 (m, 10, ArH and =CHAr), 3.59, 3.06 (d, 3 H, NCH_3).

Anal. Calcd for $C_{17}H_{14}N_2O_5$: C, 62.6; H, 4.3; N, 8.5. Found: C, 62.7; H, 4.1; N, 8.6.

α-(N-Methyl-N-*o*-acetylaminobenzoyl)aminocinnamic Acid (XIIIb).—A solution of XIe (50 mg) in 5 ml of methanol was treated with 0.5 ml of 2 *N* sodium hydroxide and 1 ml of water and the solution was heated at reflux for 10 min. The solution was diluted with water and acidified with 5% H_3PO_4, forming a precipitate which was filtered and dried to yield 38 mg of XIIIb. Recrystallization from ethyl acetate–hexane gave material of mp 231–232° dec; uv λ_{max} 280 nm (sh) (ϵ 13,600), 245 (16,400); nmr (d_6-DMSO) δ 6.8–8.1 (m, 10, ArH, =CHAr), 3.13, 2.79 (d, 3, NCH_3), 2.12, 1.97 (d, 3, CH_3CON).

***dl*-Cyclopenin (XIIa).**—A solution of XIc (0.176 g) in 10 ml of methylene chloride was treated with 85% *m*-chloroperbenzoic acid (1.06 g) and the solution was stored at 25° for 17 days. The suspension was diluted with methylene chloride to dissolution of suspended material and washed successively with 2% sodium thiosulfate, 2% sodium bicarbonate, and sodium chloride solutions. The methylene chloride phase was dried and evaporated to a viscous residue which afforded 70 mg of *dl*-cyclopenin: mp 189–191° after crystallization. Pure *dl*-cyclopenin was obtained by recrystallization from ether–hexane solution: mp 193–195°.

Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.4; H, 4.8; N, 9.5; mol wt, 294. Found: C, 69.6; H, 4.8; N, 9.5; mol wt, 294 (mass spectroscopy).

***dl*-Isocyclopenin (XIIb).**—A solution of XId (30 mg) in methylene chloride was treated with 85% *m*-chloroperbenzoic acid (200 mg) and the solution was allowed to stand at 25° for 30 days. It was then washed successively with sodium thiosulfate, sodium bicarbonate, and sodium chloride solutions. Drying and evaporating the methylene chloride gave a residue from which 10 mg of *dl*-isocyclopenin (XIIb) was obtained after two crystallizations from ether–hexane solution. The melting point was very sensitive to the rate of heating and at 10°/min was 210–213°.

Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.4; H, 4.8; N, 9.5; mol wt, 294. Found: C, 69.3; H, 5.0; N, 9.5; mol wt, 294 (mass spectroscopy).

Conversion of *dl*-Cyclopenin (XIIa) into Viridicatin (Ia).—A suspension of 0.054 g of *dl*-cyclopenin in 5 ml of 2 *N* hydrochloric acid was heated at 87° for 3 hr. The suspension was cooled and the crystalline precipitate was filtered to yield 0.042 g (97% yield) of crude product. Recrystallization from ethanol gave viridicatin: mp 265° (lit.[5] mp 269°); uv λ_{max} 329 nm (ϵ 8300), 317 (11,200), 307 sh (9100), 285 (9100), 220 (42,800).

Anal. Calcd for $C_{15}H_{11}NO_2$: C, 75.9; H, 4.7; N, 5.9. Found: C, 76.1; H, 4.7; N, 6.1.

Conversion of *dl*-Isocyclopenin (XIIb) into Viridicatin (Ia).—A suspension of 1.0 mg of XIIb in 1 ml of 2 *N* hydrochloric acid and 1 ml of ethanol was heated at reflux temperature for 3 hr. Evaporation of solvent and washing of the residue with water afforded viridicatin.

Reaction of Cyclopenin with Thiourea.—A solution of 5 mg of cyclopenin and 2.5 mg of thiourea in 2 ml of absolute butanol-1 was heated at 80–90° for 7 days. The solvent was evaporated and the residue was chromatographed by preparative tlc. The uv fluorescent band was eluted with methanol and evaporation of the methanol gave a white crystalline product (XIa–XIb): mp 232–235°; uv λ_{max} 230 sh, 267, and 319 nm; nmr (d_6-DMSO) δ 7.1–7.8 (m, 10, ArH and NH), 2.83 (d, 3, NCH_3).

Anal. Calcd for $C_{18}H_{16}N_4O_2S$: C, 61.3; H, 4.6; N, 15.9; mol wt, 352. Found: C, 60.9; H, 4.8; N, 16.6; mol wt, 352 (mass spectroscopy).

Phenylglycidanilide.—To 1 g (4.48 mmol) of *trans*-cinnamanilide[18] in 75 ml of chloroform was added 1.72 g (10 mmol) of *m*-chloroperbenzoic acid and the solution was allowed to stand at room temperature for 18 hr. The reaction mixture was extracted several times with 50 ml of 5% sodium bicarbonate solution followed by one extraction with 50 ml of water. The resultant red organic phase was dried over sodium sulfate and filtered, and the solvent was removed by evaporation. This left a red oil which was dissolved in a minimum of warm ethanol; water was added until the solution became cloudy. Cooling produced crystals and several crops were obtained by the further addition of water to give a total of 0.5 g (47%) of the epoxide: mp 142° (lit.[37] mp 142°).

Reaction of Phenylglycidanilide and Thiourea.—A mixture of 100 mg (0.7 mmol) of phenylglycidanilide, 53.6 mg (0.7 mmole) of thiourea, and 10 ml of absolute ethanol was heated on a steam bath for 4 hr. Water was added until the solution became cloudy, and storage in a refrigerator gave crystals, 84 mg (90%), mp 151–152°, identical with *trans*-cinnamanilide.

Registry No.—VIc, 19581-62-5; Xa, 5118-94-5; Xb, 3415-35-8; Xc, 1015-77-6; XIa, 19581-63-6; XIb, 19553-21-0 XIc, 19113-24-7; XId, 19553-22-1; XIe, 19553-23-2; XIf, 19614-24-5; *l* XIIa, 10088-76-3; *dl* XIIa, 19357-57-4; *dl* XIIb, 19553-26-5; XIIIa, 19543-44-3; XIIIb, 19600-43-2; ethyl *o*-nitrobenzoylsarcosinate, 19543-45-4.

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