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TOTAL SYNTHESIS OF ANTIBIOTIC INDOLMYCIN AND ITS STEREOISOMERS*

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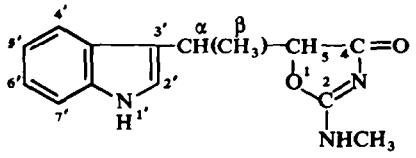
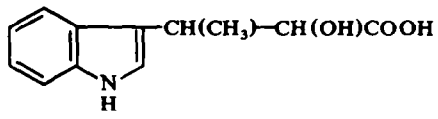
Abstract—The total synthesis of the antibiotic indolmycin [(−)-2-methylamino-5-α-(3′-indolyl)ethyl-2-oxazolin-4-one] and its stereoisomers via diastereoisomeric α-hydroxy-β-(3-indolyl)butyric esters has been described. Various 2,4-oxazolidinedione derivatives—analogues of indolmycin—were also obtained. The configuration of indolmycin was confirmed by the NMR spectra. Indolmycin in solution is a mixture of two conformers of an enaminoform with a restricted rotation around the C—NHMe bond.

The bacteriostatic and chemotherapeutic activity of (±)-indolmycin shows that the antibiotic is highly effective *in vitro* and *in vivo* against staphylococci producing penicillinase and staphylococci which are polyresistant. (−)-Indolmycin *in vitro* is twice as active as its racemate.

INDOLMYCIN elaborated by *Streptomyces albus* strain BA-3972 (isolated from an African soil) exhibits antibacterial activity against staphylococci, resistant to antibiotics used in medical practice.[4,5] It has been proved that it is (−)-2-methylamino-5-α-(3′-indolyl)ethyl-2-oxazolin-4-one [(−)-Ia]. Indolmycin in an alkaline medium readily epimerizes to dextrorotatory isoindolmycin [(+)-Ib], which is its C_5-diastereoisomer and which has no antibacterial activity.[6,7]



Ia, b



IIa, b

Both (−)-Ia and (+)-Ib when hydrolysed by alkali give α- and β-indolmycinic acids [(−)-IIa and (−)-IIb] which are epimeric at the α-atom. It has been shown by chromatography that condensation of α-indolmycinic ester, obtained from the natural indolmycin, with N,N′-dimethylguanidine yields among other products (−)-Ia and (+)-Ib. (±)-α-Hydroxy-β-(3-indolyl)butyric acid, m.p. 170° was obtained starting from ethyl *trans*-2,3-epoxybutyrate and indole in the presence of $SnCl_4$.

* See 1, 2 and 3 for preliminary communication.

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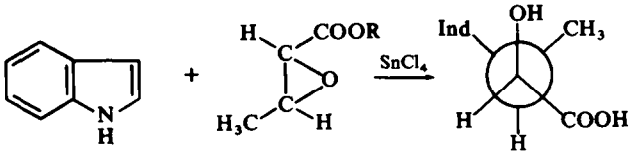
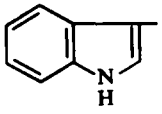
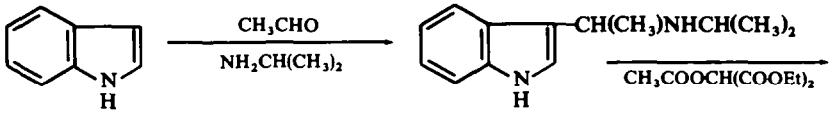
This acid was considered to be racemic α-indolmycinic acid. Its configuration III is consistent with the general mechanism of the epoxide ring opening.[7]



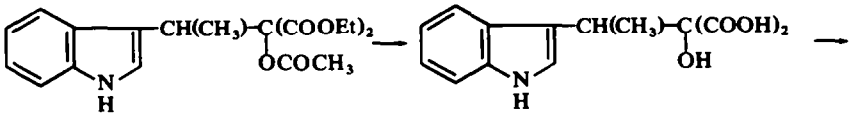
III

Here and below Ind = 

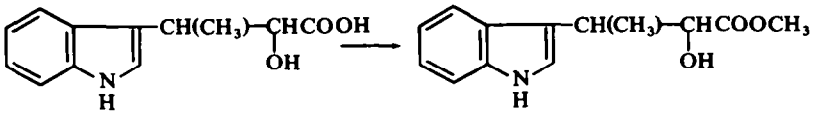
The following scheme has been used to synthesize the racemic indolmycinic acids:



IV



V VI

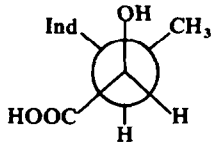


IIa, b VIIa, b

N-[α-(3-Indolyl)ethyl]-N-isopropylamine (IV), obtained from indole and ethylidenisopropylamine, reacted in the presence of sodium with acetoxymalonic ester yielding ethyl α-carbethoxy-α-acetoxy-β-(3-indolyl)butyrate (V). Saponification with alcoholic alkali yielded the substituted tartronic acid VI, which was decarboxylated to a mixture of diastereoisomeric acids which could not be separated either by crystallization or chromatography. However, using ascending paper chromatography (in a mixture of dichloroethane–acetic acid–water, 3 : 1 : 1, lower layer, time of chromatography—24 hr; system A) two spots with close R_f values were obtained. The monoacids II, when treated with CH_2N_2, gave a mixture of racemic α- and β-indol-

mycinic methyl esters, which when crystallized, yielded VIIa, m.p. 87·5–88·5° and VIIb, m.p. 94–95°. The structure of these esters* was proved by their mass-spectra.[2]

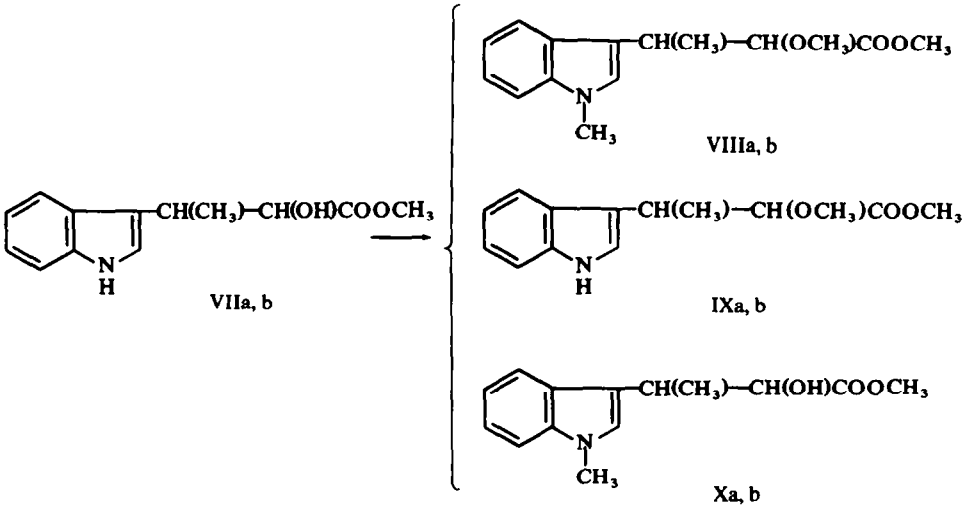
The saponification of esters VIIa and VIIb gave (±)-indolmycinic acid, m.p. 169–170° (IIa) and (±)-indolmycinic acid, m.p. 152–154° (IIb) respectively. As the m.p. of IIa coincides with that of acid III, the configuration III may be assigned to IIa and the configuration XII to IIb.



XII

The acid, m.p. 152–154° (IIb) was resolved into its optical antipodes by crystallization of its (+)-α-phenylethylamine salts. The acid, m.p. 141–142° and $[\alpha]_D^{25}$ −37·2° [(−)-IIb] was isolated from the less soluble salt; the more soluble salt yielded the acid, m.p. 140–141° and $[\alpha]_D^{25}$ +34·7° [(+)-IIb]. Natural β-indolmycinic acid melts at 142–143° and has $[\alpha]_D^{25}$ −36°. Thus, it was proved that IIb, m.p. 152–154°, is racemic β-indolmycinic acid and consequently IIa, m.p. 169–170° is racemic α-indolmycinic acid. The earlier assumption,[7] that α-indolmycinic acid has the configuration III, and β-indolmycinic acid—XII, has been confirmed.

(−)- or (+)-β-Indolmycinic acids, when treated with CH_2N_2, yielded the corresponding methyl esters [(−)-VIIb and (+)-VIIb]. The condensations of indolmycinic



* Methylation of VIIa or VIIb, after Kuhn (by the action of an excess of MeI and Ag_2O in dimethylformamide) gave a complex mixture, which was separated by chromatography.

VIIIa was separated from the mixture of IXa and Xa, and VIIIb was separated from the mixture of IXb and Xb.

The saponification of VIIIa or VIIIb resulted in stereoisomeric α-methoxy-β-(1-methyl-3-indolyl)butyric acids (XIa and XIb).

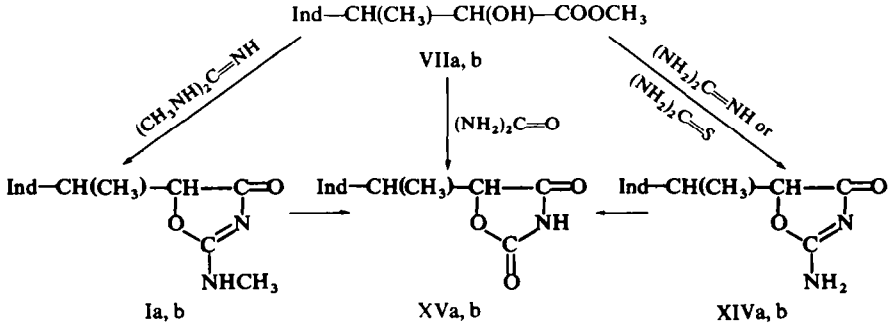
esters [(±)-α-(VIIa), (±)-β-(VIIb)], with N,N'-dimethylguanidine yielded (±)-indolmycin and (±)-isoindolmycin from α-ester (VIIa) as well as from β-ester (VIIb). The epimerization at C_5 of indolmycin and of other derivatives of oxazolidindion-2,4 occurs readily in alkaline media; but the indolmycinic acids and their noncyclic derivatives do not epimerize to any extent under these conditions. In addition to Ia and Ib, the reaction mixture contained the initial ester (VIIa or VIIb), the corresponding acid (IIa or IIb) and the methylamide of the appropriate acid (XIIIa or XIIIb) (as determined by paper chromatography in system A). The mixture of Ia and Ib was separated from the initial and collaterally formed substances by chromatography on a column with hydrated silicic acid. The process was checked by TLC on hydrated silicic acid in ether. In most cases, however, chromatography on a column was used only for purifying the mother liquors, while the basic portions of Ia and Ib were obtained without resorting to chromatography.

As both α- and β-indolmycinic esters form a mixture of Ia and Ib, there was no need to separate these esters to obtain racemic indolmycin. The following method proved to be the most convenient. The mixture of monoacids, obtained after decarboxylation of VI, was boiled with methanol and sulphuric acid, and the mixture of VIIa and VIIb was condensed with N,N'-dimethylguanidine hydroiodide in an alcohol solution of NaOEt yielding a mixture of Ia and Ib. These were separated by crystallization from acetone or methanol, the ratio Ia:Ib being ca. 1:1. Paper chromatography in 2% K_2HPO_4 (system B) was applied to control the separation.

A mixture of (−)-Ia and (+)-Ib was obtained from the methyl ester of (−)-β-indolmycinic acid [(−)-VIIb]. The diastereoisomers were separated by crystallization in acetone and methanol. The separation was checked by paper chromatography in system B. The mixture of (+)-Ia and (−)-Ib obtained from (+)-β-indolmycinic ester [(+)-VIIb] was likewise separated by crystallization.

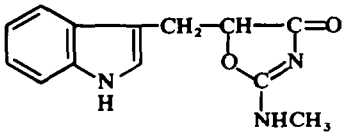
A mixture of racemic epimeric 2-amino-5-α-(3'-indolyl) ethyl-2-oxazoline-4-ones (XIVa, b) was obtained by the condensation of VIIa or VIIb esters with guanidine salts or thiourea in the presence of NaOEt. The mixture was separated from the initial and collaterally formed substances by chromatography on a column with hydrated silicic acid. It was possible to separate XIVa and XIVb by crystallization in ethyl acetate, the process was controlled by paper chromatography in system B.

Condensation of VIIa or VIIb with urea in the presence of NaOEt produced in each case a mixture of racemic 5-α-(3'-indolyl)ethyloxazolidin-2,4-diones (XVa and XVb), which were separated by crystallization from chloroform. Compounds XVa and XVb have different R_f values (paper chromatography in system B).



Compound XVa was also obtained by acid hydrolysis of Ia and amino-oxazolinon XIVa; and XVb was obtained by acid hydrolysis of Ib and amino-oxazolinone XIVb. Thus, the configuration of XIVa and XVa coincides with that of indolmycin and the configuration of XIVb abd XVb with that of isoindolmycin.

Condensation of methyl 3-indolyllactate with N,N'-dimethylguanidine yielded C-desmethylindolmycin—5-skatyl-2-methylamino-2-oxazoline-4-on (XVI).



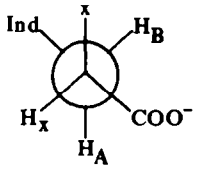
XVI

The configuration of α- and β-indolmycinic acids (IIa and IIb) also has been deduced by comparison of its NMR spectra with those of tryptophane and 3-indolyllactic acid. The protons of the CH_2 group of tryptophane or 3-indolyllactic acid show in alkaline media the AB part of the ABX spectral pattern with J_{vic} given in Table 1.

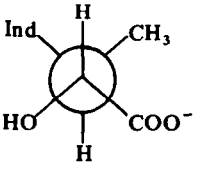
TABLE 1

J_{vic}	Tryptophane	3-Indolyllactic acid
J_{AX}	5 c/s	4·2 c/s
J_{BX}	8 c/s	7·7 c/s

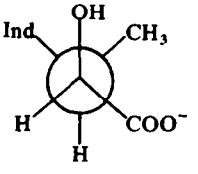
These data suggest, that in alkaline media tryptophane and 3-indolyllactic acid have the predominant conformations with *trans*-position of COO^- and *Ind* fragments, the proton B being in *trans*-position and the proton A—in *gauche* position to the proton X (XVII).



XVII $x = NH_2$, OH



XVIII

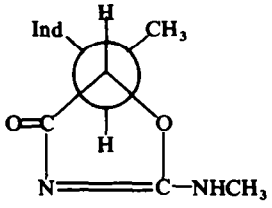


XIX

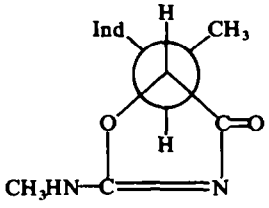
Similarly, for α- and β-indolmycinic acids (IIa and IIb) in alkaline media the quota of conformers with *trans*-position of COO^- and *Ind* groups should increase. One can suggest therefore, that for IIa and IIb in alkaline solution, the constant J_{12} should be greater for the isomer for which the conformer with *trans*-position of COO^- and *Ind*-groups has the vicinal protons in *trans*-position (XVIII). As can be seen from the Table 2, α- and β-indolmycinic acids have practically identical J_{12} in neutral

media, but in alkaline solution only J_{12} for IIb increases. Thus, configuration XVIII may be ascribed to IIb and configuration XIX to IIa; i.e. the configurations deduced from NMR studies are in accordance with assumption based on synthetic methods.

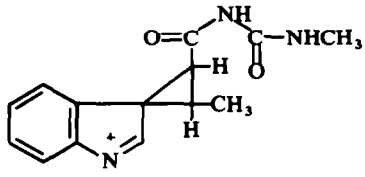
American authors proposed that the hydrogen atoms at C_5 and C_α in indolmycin and isoindolmycin are in *trans*-position in the least hindered conformations (XX and XXI).[7]



XX



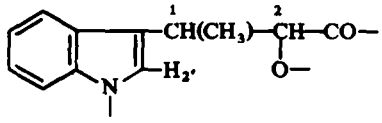
XXI



XXII

The isomerization of the carbon skeleton by acid degradation of Ia, may be explained by postulating cleavage of the C_5—O bond and formation of the intermediate XXII. As Ib does not rearrange with migration of the indole moiety to any appreciable

TABLE 2. CHEMICAL SHIFTS AND SPIN COUPLING CONSTANTS IN COMPOUNDS OBTAINED



Compound	δ (ppm)							$J_{1,2}$	Solvent
	1	2	CH_3	NCH_3	$NHCH_3$	OCH_3	2″		
IIa	3·59	4·41	1·35				7·12	3·8	CD_3OD
b	3·63	4·37	1·45				7·14	3·8	
IIa	3·70	4·37	1·28				7·23	3·2	2NNaOD
b	3·68	4·42	1·55				7·31	5·1	
VIIIa	3·60	4·44	1·28			3·73	6·94	3	$CDCl_3$
b	3·66	4·38	1·47			3·51	7·01	3	
XIa	3·62	4·02	1·38	3·31		3·72	6·94	3·8	$CDCl_3$
b	3·68	3·96	1·40	3·43		3·68	6·94	4·2	
XVa	3·74	5·17	1·39				7·21	3	CD_3COCD_3
b	3·79	5·15	1·53				7·20	3	
XIVa	3·77	5·00	1·38				7·14	2·5	CD_3OD
b	3·77	4·97	1·63				7·11	3	
Ia	4·01	5·12	1·42		2·91			3	pyridin
	4·01	5·16	1·48		2·80			3	
b	3·92	5·00	1·57		2·73			4	
	3·92	5·00	1·63		2·80			4	

extent, it was proposed that the C-*Ind* and C_5—O_1 bonds are in *trans*-position in Ia in the least hindered conformation. Hence, configuration XX was attributed to Ia, and configuration XXI to Ib, i.e. the configuration of α-indolmycinic acid was attributed to indolmycin, and that of β-indolmycinic acid to isoindolmycin.[7]

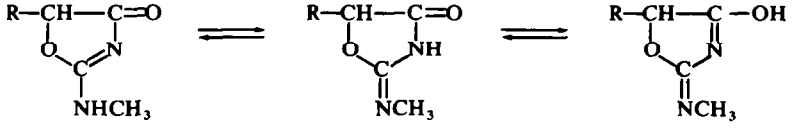
The NMR spectra of the compounds obtained has confirmed the assumption that indolmycin belongs to the IIa series, and isoindolmycin to the IIb series. However, the conclusions drawn by the American authors about the preferable conformation of Ia and Ib seem to be doubtful.

Table 2 presents the values of the chemical shifts and the spin coupling constants for the compounds obtained. It follows from Table 2 that the constants $J_{1,2}$ are small in every case (about 3·5 c/s). Hence, the conformer with a *trans*-position of protons is not predominant for any of the compounds in the series Ia, Ib, IIa and IIb (at any rate in neutral media). Evidently stabilization of the conformers occurs not on account of the *trans*-disposition of the largest substituents, but as a result of interaction between certain substituents. It is natural to assume that in the indolmycin and isoindolmycin series and in the series of α- and β-indolmycinic acids, i.e. among the compounds possessing an Ind—$CH(CH_3)$—CH(CO—)(O—) fragment, the predominant conformer is determined by the interaction of similar substituents. It might therefore be expected that the relationship between the chemical shifts of similar protons or groups of protons in epimers should be approximately the same for all the compounds. Indeed, the signals of the Me-group protons of IIb and its non-cyclic derivatives are always in a lower field than those of the Me group protons of IIa and its corresponding derivatives. In the series of Ib and its derivatives the signals of the Me group protons are always in a lower field than those of the Me group protons of Ia and its corresponding derivatives. Indolmycin therefore has the configuration of α-indolmycinic acid, and isoindolmycin—that of β-indolmycinic acid.

This is also proved by studying the chromatographic mobility of the compounds. In system B, for each pair of epimers, the derivative with a configuration of indolmycin has a lesser mobility than the corresponding derivative with a configuration of isoindolmycin: likewise, the derivative with a configuration of α-indolmycinic acid has a lesser mobility than its isomer with a configuration of β-indolmycinic acid.

The tendency of Ia to rearrange in acid media probably depends on the structure of intermediate XXII. The substituents (CONH— and CH_3) of the intermediate, derived from Ia are not ecliptic; in the case of Ib the spiroindolenine intermediate should have these large substituents in ecliptic (*cis*) position.

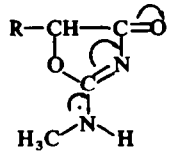
An enamino-imino (XXIII ⇄ XXIV) and lactim-lactam (XXIV ⇄ XXV) tautomeric equilibrium is possible for the derivatives of 2-methylamino-2-oxazolin-4-one:*



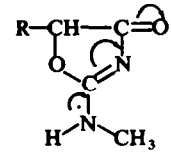
XXIII XXIV XXV

* The structures without hydrogen atom at C_5 are not considered, since there are no forms in NMR spectra without a proton at C_5.

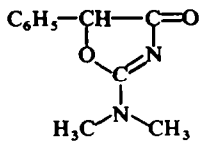
In addition, the enaminoform (XXIII) may exist as two conformers (XXIII-*cis* and XXIII-*trans*) in the case of a restricted rotation around C—NHMe bond:



XXIII-*cis*

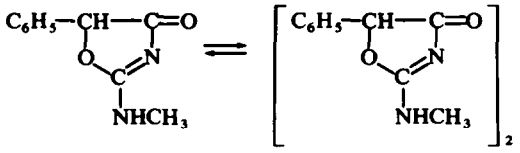


XXIII-*trans*



XXVI

The non-equivalence of the protons of two Me groups in compound XXVI has been noted,[8] and it was assumed that for 5-phenyl-2-methylamino-2-oxazolin-4-one, a monomer-dimer equilibrium (XXVII ⇄ XXVIII) exists in the solution:[8]



XXVII XXVIII

In the NMR spectra of indolmycin or isoindolmycin, two signals located near one another on the scale of chemical shifts belong to each proton (or group of equivalent protons) (see Table 2 and Fig. 1). This proves that each of the epimers exists in two forms, with relative concentrations ca. 1:2. The lifetime of each of the forms at room temperature is at least 1 sec. It is possible to observe the splitting of the N—Me signal of one of the forms (J_{Me-NH} = 5 c/s) in pyridine, which points unambiguously to the structure XXIII for this form.

In acid ($CD_3OD + D_2SO_4$) or in alkaline ($CD_3OD + NaOCD_3$ or CD_3OD + NaOH) media, both Ia and Ib likewise, exist in two forms, having approximately the same ratios as in a neutral medium. This cannot be explained by the existence of an enamino-imino equilibrium, since protonation of tautomeric enamino- and imino-forms leads to one and the same structure (the same as the elimination of a proton). The existence of two forms in alkaline and acid media cannot be explained in terms of one form being a monomer, and the other a dimer, since it is difficult to assume that, despite the ionization of the molecule, the ratio between monomer and dimer practically undergoes no change. During ionization in alkaline and acid media there is considerable change in chemical shift of the proton at C_5 as compared with the chemical shift in a neutral medium—e.g. one of the forms of indolmycin in $CD_3OD\delta_{C_5-H}$ 4·89 ppm, in $CD_3OD + NaOCD_3$ δ_{C_5-H} 4·61 ppm, in $CD_3OD + D_2SO_4$ δ_{C_5-H} 5·40 ppm; $\Delta\delta$ upon ionization is similar for both forms of indolmycin (or isoindolmycin). The absence of any concentration-dependence (in pyridine) is also contrary to monomer-dimer equilibrium.

Thus, the NMR spectra of indolmycin (and isoindolmycin) in various solvents at different concentrations indicates that Ia (or Ib) exists in solutions as a mixture

of conformers of enaminoform XXIII (*cis* and *trans*) with a restricted rotation around the C—NHMe bond.

The difference in the nature of the signal from the N—Me group (a doublet for the first form and a singlet for the second), may be accounted for by the rate of exchange between the proton of the Me—NH group and the other exchangeable protons. This, in turn, depends on the accessibility of such a proton, i.e. on its disposition in relation to the rest of the molecule.

As might be expected, both diastereoisomers of 2-amino-5-α-(3'-indolyl)ethyl-2-oxazoline-4-ones (XIVa and XIVb) exist in one form, since both the conformers are identical in this case.

Activity of the compounds synthesized has been studied *in vitro* and *in vivo*. The minimal bacteriostatic concentrations of (±)-indolmycin against staphylococci (8 strains) ranged from 0·125 γ/ml to 4 γ/ml. The racemic indolmycin was effective against staphylococci producing penicillinase and staphylococci which are polyresistant. In the experiments with streptococci, pneumococci, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Salmonella Gärtneri*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, the bacteriostatic activity of (±)-indolmycin was 31–250 γ/ml and with *Mycobacterium tuberculosis* it was 8–16 γ/ml. In the experiments with staphylococci and *Escherichia coli*, (−)-indolmycin proved to be twice as active as the racemate. (+)-Indolmycin, (±)-isoindolmycin and its optical isomers, N-desmethylindolmycin (XIVa), C-desmethylindolmycin (XVI) and oxazolidindion (XVa), were slightly active against staphylococci. (±)-Indolmycin was well tolerated by mice when administered in single oral doses of 500 mg/kg and had a good chemotherapeutic effect in doses of 50 to 500 mg/kg against sepsis in mice caused by polyresistant staphylococci. In parallel experiments ampicillin, chloramphenicol and erythromycin were less active than (±)-indolmycin or not active at all.

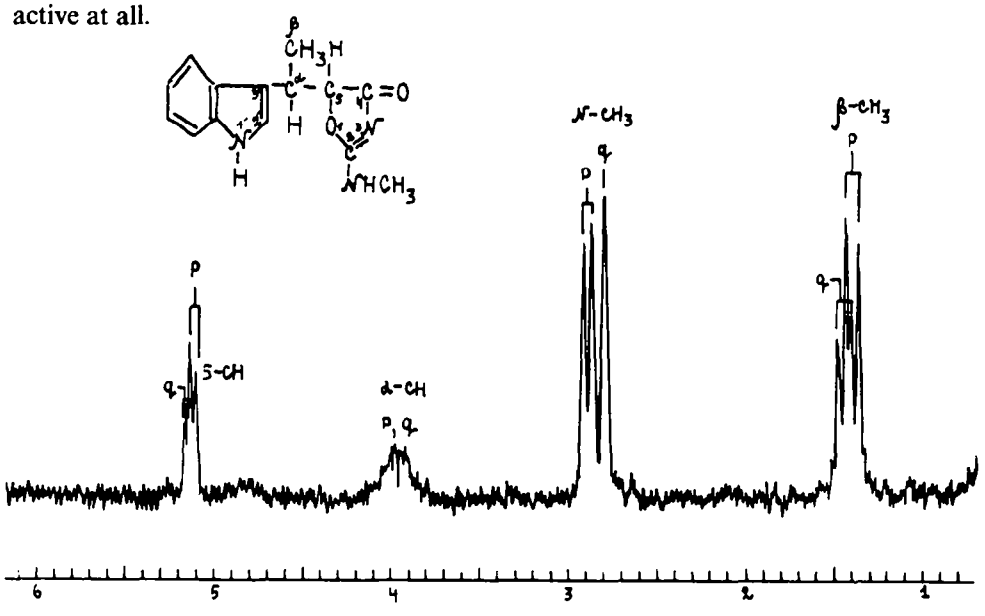


FIG. 1 NMR spectrum of indolmycin (Ia) in pyridine. p = protons of the first form of Ia, q = protons of the second form of Ia.

EXPERIMENTAL

The NMR spectra were measured with TMS as internal standard using an JNM-4H100 instrument possessing operating frequency of 100 mc. IR-spectra were photographed with UR-10 instrument.

N-(α-(3-Indolyl)ethyl)-N-isopropylamine (IV). Ethylidenisopropylamine (30·8 g) in 65 ml C_6H_6 was added dropwise to a stirred soln of indole (39 g) in 200 ml glacial AcOH at +10–+5°.[9] The mixture was kept in a refrigerator overnight, then 675 ml water and 67·5 ml C_6H_6 were added with stirring. The benzene layer was separated, the water layer extracted with benzene (68 ml × 3) and 70 ml toluene were added to the water layer and 20% NaOH (about 400 ml) was added dropwise at 0–+10° up to pH 12. After refrigeration for 3–4 hr, the ppt was filtered off, washed with cold water yielding 41·7 g crude IV (62%), m.p. 109–110°.

α-Carboxy-α-hydroxy-β-(3-indolyl)butyric acid (VI). Crude IV (106 g), acetoxymalonic ester (200 g) and Na (4 g) were boiled in dry xylene (940 ml) under N_2 until evolution of isopropylamine ceased (about 20 hr). Upon cooling, 8 ml alcohol were added, the xylene was evaporated *in vacuo*, and a soln of NaOH (208 g) in water (264 ml) and alcohol (1060 ml) was carefully added. The mixture was stirred for several hr and kept at room temp overnight. The disodium salt VI was filtered off, washed with ether (about 200 ml), and then dissolved in water and acidified with HCl to pH 1. If the acid is added slowly, the monosodium salt sometimes precipitates, but dissolves upon further acidification. VI was extracted with EtOAc (300 ml × 4), the extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was washed with dichloroethane to yield 110 g of VI (80%), m.p. 151·5–152° from dichloroethane. (Found: C, 59·05; H, 5·22; N, 5·29. $C_{13}H_{13}NO_5$ requires: C, 59·28; H, 4·98; N, 5·32%. R_f 0–0·2, system A).

Methyl α-carbomethoxy-α-hydroxy-β-(3-indolyl)butyrate. 21·7 g of VI were added in portions to a stirred ether soln of CH_2N_2. The soln was filtered, the ether was evaporated yielding 24·6 g crude diester. This was dissolved in EtOAc, washed with a 2% $NaHCO_3$ aq and dried over Na_2SO_4. The soln was evaporated and the residue recrystallized from benzene and CCl_4, then thoroughly dried in *a vacuo* (desiccator) m.p. 86·5–87°. (Found: C, 62·34; H, 5·67; N, 4·84. $C_{15}H_{17}NO_5$ requires: C, 61·82; H, 5·84; N, 4·71%).

Mixture of racemic α- and β-indolmycinic acids (IIa *and* IIb). Diacid VI (15 g) was ground to a powder and boiled in xylene (1·5 l.) under N_2 until the ppt was completely dissolved (30–40 min). The soln was slightly cooled and after adding charcoal, it was boiled for 10 min; then filtered, and the filtrate cooled for 24 hr, yielding 11·6 g (93%) of a mixture of monoacids II with m.p. 131–133°. (Found: C, 65·69; H, 5·85; N, 6·52. $C_{12}H_{13}NO_3$ requires: C, 65·74; H, 5·98; N, 6·39%); IR spectrum: 3460, 3400, 1710, 1730 cm^{-1} (Nujol). R_f 0·52 and 0·62 (system A), and 0·63 and 0·72 (system B).

(±)-Indolmycin and (±)-isoindolmycin (Ia *and* Ib). 13·2 g of monoacids II, 300 ml abs MeOH and 20 ml conc H_2SO_4 were boiled for 17 hr, 1 l. ice water was added and the mixture was extracted with EtOAc. The EtOAc extract washed with a 2% $NaHCO_3$ aq, then with water, and dried over Na_2SO_4. The soln was evaporated *in vacuo*, 12·8 g N,N'-dimethylguanidine hydroiodide and 35 ml 6N NaOEt were added to the residue and the mixture boiled for 10 hr. It was then cooled, 80 ml ice water added, acidified with 20% AcOH to pH 5 and extracted with EtOAc (40 ml × 6). The extract was washed with a 2% $NaHCO_3$ aq, dried over Na_2SO_4 evaporated and the residue again dissolved in EtOAc. After scratching and cooling, a ppt (a mixture of Ia and Ib) was formed, 6·8 g (43·8%); R_f 0·45 and 0·59 (system B). The mixture of Ia and Ib was dissolved in hot MeOH and the soln kept in a refrigerator overnight. A crystalline ppt of mostly Ib was obtained. The MeOH mother liquor was evaporated to dryness, dissolved while heating in a minimum amount of acetone, and crystalline commercial Ia was filtered off after a few hr. The mother liquor was evaporated, the residue was dissolved in MeOH, and the procedure repeated. When crystallizing from methanol, it was desirable to add a priming of Ib, and when crystallizing from acetone, a priming of Ia. The portions of commercial Ia or Ib were collected and recrystallized from acetone or MeOH respectively to yield 2·8 g of Ia (18%) and 2·3 g of Ib (15%). The purity of the substances was checked by paper chromatography in B system. (±)-Indolmycin is a colourless substance, m.p. 183·5–184° from acetone, soluble by heating in water, alcohol and acetone, and insoluble in non-polar solvents. (Found: C, 65·66; H, 6·03; N, 16·19. $C_{14}H_{15}N_3O_2$ requires: C, 65·35; H, 5·88; N, 16·33%); IR spectrum: 3340, 3080, 2990, 2950, 2900, 1745, 1640, 1550 cm^{-1} (KBr); R_f 0·89 (system A), 0·45 (system B). An orange picrate of Ia was obtained in abs alcohol, m.p. 162–163° dec. (Found: C, 49·51; H, 3·85; N, 17·06. $C_{14}H_{15}N_3O_2 \cdot C_6H_3N_3O_7$ requires: C, 49·39; H, 3·73; N, 17·28%).

(±)-Isoindolemycin is a colourless substance, m.p. 214·5–215° from acetone, less soluble than Ia in alcohol and acetone, and more soluble in water. (Found: C, 65·57; H, 6·07; N, 16·30. $C_{14}H_{15}N_3O_2$ re-

quires: C, 65·35; H, 5·88; N, 16·33%); IR spectrum: 3440, 3220, 3150, 3080, 2990, 2950, 2930, 2900, 1740, 1650, 1620, 1560 cm^{-1} (KBr); R_f 0·89 (system A), 0·59 (system B).

A yellow picrate of Ib was obtained in abs alcohol, m.p. 162–163° dec. (Found: C, 49·20; H, 3·95; N, 16·95. $C_{14}H_{15}N_3O_2 \cdot C_6H_3N_3O_7$ requires: C, 49·39; H, 3·73; N, 17·28%).

Methyl esters of α- *and* β-*indolmycinic acids* (VIIa *and* VIIb). A mixture of IIa and IIb (3·5 g) was added in small portions with stirring to an ether soln of CH_2N_2. The soln was evaporated *in vacuo*, 25 ml benzene were added and after cooling scratched (if the experiment was repeated, priming was used). A few hr later, the ppt (1·4 g of VIIa) was filtered off. Crystals of VIIb were added to the mother liquor and, when cooled, 1·57 g of VIIb were obtained. To produce a VIIb priming, pet ether was added to the mother liquor benzene soln and the ppt obtained after scratching and cooling was recrystallized from CCl_4. After separation of VIIb, the mother liquor was evaporated to 10 ml and about 0·1 g more of VIIa was obtained, and from its mother liquor another 0·2 g of VIIb. All the fractions of VIIa and of VIIb were combined respectively and recrystallized from benzene or CCl_4 to yield 1·37 g of VIIa (37%) and 1·5 g of VIIb (41%). VIIa melts at 88–89° from benzene. (Found: C, 66·83; H, 6·34; N, 6·06. $C_{13}H_{15}NO_3$ requires: C, 66·92; H, 6·48; N, 6·01%); IR spectrum: 3500, 3390, 1720 cm^{-1} (Nujol) VIIb, m.p. 94–95° from benzene, CCl_4. (Found: C, 66·92; H, 6·57; N, 6·02. $C_{13}H_{15}NO_3$ requires: C, 66·92; H, 6·48; N, 6·01%); IR spectrum: 3510, 3370, 1730 cm^{-1} (Nujol).

VIIb sometimes forms crystals with m.p. 66·5–67·5°, but this modification changes into one with m.p. 94–95° when crystallized from benzene. A mixed sample of VIIa with any of the VIIb forms produces a drop of the m.p.

(±)-α-*Indolmycinic acid* (IIa). A mixture of VIIa (37 g), EtOH (210 ml) and 10% KOH (100 ml) was boiled for 2 hr, the alcohol was evaporated, and the water residue was extracted with EtOAc and acidified with dil HCl. IIa was extracted with EtOAc (25 ml × 5); the extract dried over $NaSO_4$ and evaporated *in vacuo*, the residue was washed with dichloroethane to yield 30·4 g (87·5%) of IIa, m.p. 169–170° from benzene and water. (Found: C, 66·51; H, 6·06; N, 6·44. $C_{12}H_{13}NO_3$ requires: C, 65·74; H, 5·98; N, 6·39%); IR spectrum: 3400–3440, 1730 cm^{-1} (Nujol); R_f 0·52 (system A), 0·63 (system B).

(±)-β-*Indolmycinic acid* (IIb). In a similar way, saponification of VIIb yielded IIb with m.p. 152–154° from a benzene–acetone mixture, 3:1. (Found: C, 65·66; H, 5·99; N, 6·34. $C_{12}H_{13}NO_3$ requires: C, 65·74; H, 5·98; N, 6·39%); IR spectrum: 3460, 3420, 1710 cm^{-1} (Nujol); R_f 0·62 (system A), 0·72 (system B).

A mixed sample of IIa and IIb had a m.p. about 130°.

Salts of β-*indolmycinic acid and* (+)-α-*phenylethylamine.* (−)- *and* (+)-β-*Indolmycinic acids* ((−)-IIb, (+)-IIb). (+)-α-Phenylethylamine (14·7 g) in acetone (7 ml) was added to a soln of IIb (26·7 g) in acetone (260 ml). The mixture was kept for 3 hr in a refrigerator, the ppt was filtered off and washed with cold acetone to yield 34·1 g (86·8%) of a mixture of diastereoisomeric salts m.p. 152–154°. The mixture was separated by fractional crystallization from acetone to yield a slightly soluble salt with m.p. 170–171° and a salt more readily soluble in acetone with m.p. 167·5–168·5°. 10 g of the salt with m.p. 170–171° was dissolved in water and acidified with dil HCl to pH 2; the precipitated acid was filtered off and the mother liquor extracted with benzene, yielding 7·7 g of (−)-β-indolmycinic acid, m.p. 141–142° from dichloroethane and water, $[\alpha]_D^{25}$ −37·2° (c 1·8 in MeOH).

In a similar way, the salt with m.p. 167·5–168·5° yielded (+)-β-indolmycinic acid, m.p. 140–141° from dichloroethane and water, $[\alpha]_D^{25}$ +34·7° (*c* 1·8 in MeOH).

Methyl ester of (−)-β-*indolmycinic acid* ((−)-VIIIb). While stirring, (−)-β-indolmycinic acid (4 g) was added to an ether soln of CH_2N_2. The mixture was stirred for 30 min, and the soln filtered and evaporated to dryness. The residue was recrystallized from benzene, yielding 3·88 g of (−)-VIIb (91·3%) with m.p. 124–126° from benzene; $[\alpha]_D^{25}$ −61·1° (*c*, 1·8 in MeOH). (Found: C, 66·83; H, 6·59; N, 5·77. $C_{13}H_{15}NO_3$ requires: C, 66·92; H, 6·48; N, 6·01%).

Methyl ester of (+)-β-*indolmycinic acid* ((+)-VIIb). This was obtained in a similar way from (+)-IIb, m.p. 124–126° from benzene, $[\alpha]_D^{25}$ +63·8° (*c*, 1·8 in MeOH). (Found: C, 67·39; H, 6·53; N, 5·85. $C_{13}H_{15}NO_3$ requires: C, 66·92; H, 6·48; N, 6·01%).

(−)-*Indolmycin and* (+)-*isoindolmycin* ((−)-Ia and (+)-Ib). (−)-VIIb (1·54 g), N,N'-dimethylguanidine hydroiodide (1·41 g) and 6·9 ml 1·91 N EtONa were boiled for 10 hr. The soln was evaporated, 100 ml water was added to the cooled residue and it was acidified with dil AcOH to pH 5. The mixture was extracted with EtOAc (25 × 5); the extract was washed with a 2% $NaHCO_3$ aq, then with water, dried over Na_2SO_4; evaporated *in vacuo* to a volume of 5–10 ml to yield a ppt, m.p. 234–238°. After further evaporation of the mother liquor, a ppt, m.p. 208–210° was obtained. The substance, m.p. 234–238° was recrystallized from an acetone–MeOH yielding 0·15 g of (+)-isoindolmycin (8·9%) with m.p. 240–242°, $[\alpha]_D^{25}$ +47°

(*c*, 0·5 in MeOH). (Found: C, 65·21; H, 5·69; N, 16·33. Calc. for $C_{14}H_{15}N_3O_2$: C, 65·35; H, 5·88; N, 16·33%); IR spectrum: 3440, 3220, 3140, 3065, 1740, 1655, 1625 cm^{-1} (Nujol). The values of R_f coincide with those found for (±)-isoindolmycin. Natural isoindolmycin: m.p. 242–245°, $[\alpha]_D^{25}$ +47° (*c* 1 in MeOH).[7]

The substance, m.p. 208–210° was recrystallized from acetone–MeOH to yield (−)-indolmycin (0·18 g, 10·6%), m.p. 211–213°, $[\alpha]_D^{25}$ −213° (*c*, 1·65 in MeOH). (Found: C, 65·08; H, 5·84; N, 16·22. Calc. for $C_{14}H_{15}N_3O_2$: C, 65·35; H, 5·88; N, 16·33%). Natural indolmycin: m.p. 212°,[7] $[\alpha]_D^{25}$ −214° (*c* 2 in MeOH).[4,5]

IR spectrum: 3300, 3215, 3130, 3100, 3080, 1735, 1615 cm^{-1} (Nujol). The values of R_f coincide with those for (±)-indolmycin.

(+)-Indolmycin and (−)-isoindolmycin ((+)-Ia *and* (−)-Ib). (+)-Ia and (−)-Ib were produced from (+)-VIIb and N,N'-dimethylguanidine hydroiodide under similar conditions. (+)-Indolmycin, m.p. 211·5–212° from an acetone–MeOH; $[\alpha]_D^{25}$ +211·4° (*c*, 1·64 in MeOH). (Found: C, 65·54; H, 6·00; N, 16·45. $C_{14}H_{15}N_3O_2$ requires: C, 65·35; H, 5·88; N, 16·33%); IR spectrum coincides with that of (−)-Ia.

(−)-Isoindolmycin, m.p. 238–240° from an acetone–MeOH; $[\alpha]_D^{25}$ −46° (*c*, 0·5 in MeOH). (Found: C, 65·21; H, 5·69; N, 16·33. $C_{14}H_{15}N_3O_2$ requires: C, 65·35; H, 5·88; N, 16·33%; IR spectrum coincides with that of (+)-Ib.

Stereoisomeric oxazolidindions XVa *and* XVb. VIIa or VIIb (2·33 g), urea (0·6 g) and 1·3N NaOEt (16·2 ml) were boiled for 20 hr. If VIIa was used, a ppt of Na salt of IIa was formed, which was filtered off. The soln was evaporated, the residue dissolved in water, extracted with EtOAc and acidified with 2N AcOH. A little $CHCl_3$ was added and the mixture kept in a refrigerator overnight yielding 0·82 g of XVa, m.p. 186·5–187·2° from dichloroethane or water. (Found: C, 64·10; H, 5·11; N, 11·61. $C_{13}H_{12}N_2O_3$ requires: C, 63·92; H, 4·95; N, 11·47%); IR spectrum: 3440, 3220, 1815, 1745 cm^{-1} (Nujol); R_f 0·46 (system B), 0·83 (system A).

The $CHCl_3$ layer was separated from the filtrate, the water layer extracted with EtOAc, and the $CHCl_3$ and EtOAc extracts combined, washed with 2% $NaHCO_3$ aq and then with a 5% Na_2CO_3 aq; the Na_2CO_3 soln was acidified with AcOH and extracted with EtOAC; the EtOAc was evaporated, the residue dissolved in $CHCl_3$ and a priming of XVa was added. Cooling yielded an additional 0·18 g of XVa. The total yield of XVa was 41%. The mother liquor was evaporated and the residue dissolved in benzene, and a priming of XVb was added (obtained from hydrolysis of Ib) yielding 0·25 g (10·2%) of XVb, m.p. 153–154° from water. (Found: C, 64·22; H, 5·00; N, 11·60. $C_{13}H_{12}N_2O_3$ requires: C, 63·92; H, 4·95; N, 11·47%); IR spectrum: 3400, 3340, 1830, 1760 cm^{-1} (Nujol); R_f 0·6 (system B), 0·83 (system A).

Stereoisomeric-2-amino-2-oxazoline-4-ones (XIVa *and* XIVb). VIIa or VIIb (1·85 g), guanidine nitrate (0·96 g) and 0·8N NaOEt (10·2 ml) were boiled for 4 hr and kept at room temp overnight. If VIIa was used, a ppt of Na salt of IIa was formed, which was filtered off. The soln was evaporated, the residue dissolved in water, extracted with EtOAc, placed on a column with hydrated silicic acid and eluated with ether and then with acetone. Control was effected by TLC on hydrated silicic acid in ether. The fractions which produced a spot, R_f 0·05 were selected. They were evaporated and the residue was dissolved in EtOAc. Crystallization after several days without scratching yielded 0·52 g (27%) of XIVb with m.p. 181–182° from water. (Found: C, 64·07; H, 5·50; N, 17·20. $C_{13}H_{13}N_3O_2$ requires: C, 64·18; H, 5·39; N, 17·28%); IR spectrum: 3410, 3280, 3150, 1745, 1670 cm^{-1} (Nujol). R_f 0·57 (system B), 0·70 (system A).

The picrate of XIVb was obtained, soluble in alcohol, m.p. 152–154°. (Found: C, 48·00; H, 3·37; N, 17·00. $C_{13}H_{13}N_3O_2 \cdot C_6H_3N_3O_7$ requires: C, 48·31; H, 3·41; N, 17·39%).

From the mother liquor of XIVb, kept for several days without scratching, 0·22 g (11·5%) of XIVa were obtained, m.p. 192–193° from EtOAc. (Found: C, 64·25; H, 5·59; N, 17·46. $C_{13}H_{13}N_3O_2$ requires: C, 64·18; H, 5·39; N, 17·28%); IR spectrum: 3430, 3310, 1740, 1670 cm^{-1} (Nujol). R_f 0·43 (system B), 0·70 (system A). A red dipicrate was obtained from alcohol, m.p. 145–147° dec. (Found: C, 42·59; H, 2·65. $C_{13}H_{13}N_3O_2 \cdot 2C_6H_3N_3O_7$ requires: C, 42·80; H, 2·79%). If thiourea was used instead of guanidine nitrate, the reaction was carried out under the same conditions.

Hydrolysis of Ia, Ib, XIVa *and* XIVb. This was carried out under conditions described for natural indolemycin.[7] The fractions which did not pass into 2% $NaHCO_3$ aq but which were soluble in a 5% Na_2CO_3 aq were studied. The yields of XVa or XVb were about 30%.

Methylation of VIIa. VIIa (4 g), Ag_2O (7·7 g) and MeI (13 ml) were mixed with cooling in 50 ml dimethylformamide. After 48 hr the ppt was filtered off, washed with dimethylformamide and the soln evaporated *in vacuo*. The residue was ground with silicic acid and boiled with benzene (30 ml × 3). All the benzene extracts were evaporated to a small volume. The chromatogram of the reaction mixture on a thin layer of hydrated silicic acid in the benzene–ether 10:1 system had spots with R_f 0·9, R_f 0·73 and R_f 0·49 and a spot with R_f 0·26, corresponding to the initial VIIa. The benzene soln was placed on a column with hy-

drated silicic acid and eluated with a benzene–cyclohexane 10:1 mixture, the fractions being checked by the TLC method. The fractions with R_f 0·73 in the benzene–ether 10:1 system were selected. Evaporation yielded 2·53 g (57%) of VIIIa, m.p. 55·5–56·5 from hexane. (Found: C, 69·03; H, 7·59; N, 5·48. $C_{15}H_{19}NO_3$ requires: C, 68·94; H, 7·33; N, 5·36%); IR spectrum: 3130, 3090, 3070, 3050, 1755 cm^{-1} (Nujol). The fractions from the column with R_f 0·49 on the plate produced after evaporation 0·45 g of a IXa and Xa mixture (10·7%).

VIIIa (1·91 g) was boiled for an hr in 20 ml 50% alcohol with 1 g KOH; the alcohol was evaporated, and the water layer was washed with ether and acidified with dil HCl yielding 1·62 g (90%) of XIa, m.p. 132·5–134°. (Found: C, 67·79; H, 7·14; N, 5·72. $C_{14}H_{17}NO_3$ requires: C, 68·00; H, 6·93; N, 5·67%); IR spectrum: 3100, 1718 cm^{-1} (Nujol); R_f 0·76 (system B).

Methylation of VIIb. In a similar way, VIIIb (3·4 g, 64%) and IXb and Xb mixture (0·34 g, 6·4%) were obtained from VIIb (4·75 g), Ag_2O (10 g) and 16 ml MeI in 50 ml dimethylformamide. Saponification of VIIIb yielded 90% of XIb, m.p. 146·0–147° from water. (Found: C, 68·22; H, 6·60, N, 5·92%. $C_{14}H_{17}NO_3$ requires: C, 68·00; H, 6·93; N, 5·67%); IR spectrum: 3080, 1730 cm^{-1} (Nujol); R_f 0·83 (system B).

Methylamides of α- *and* β-*indolmycinic acids* (XIIIa *and* XIIIb). If pure VIIa was used to obtain Ia and Ib, the mother liquor was examined chromatographically after separating the Ia and Ib mixture in EtOAc. Chromatography on a thin layer of aqueous silicic acid in ether indicated to the presence of three spots: R_f 0·85 (VIIa), 0·25 and 0·05 (a mixture of Ia and Ib). The mother liquor was chromatographed on a column with aqueous silicic acid in ether, selecting the fraction with R_f 0·25. This produced XIIIa (2%), m.p. 153–154·5 from benzene. (Found: C, 67·14; H, 6·80, N, 11·88. $C_{13}H_{16}N_2O_2$ requires: C, 67·21; H, 6·94; N, 12·06%); IR spectrum: 3400, 3300, 1630 cm^{-1} (Nujol); R_f 0·57 (system B).

In a similar way, if VIIb was used to produce Ia and Ib, XIIIb was obtained from the mother liquor, m.p. 161–162° from benzene. (Found: C, 67·50; H, 6·70; N, 11·91. $C_{13}H_{16}N_2O_2$ requires: C, 67·21; H, 6·94; N, 12·06%); IR spectrum: 3400, 1630 cm^{-1} (Nujol); R_f 0·70 (system B).

5-Skatyl-2-methylamino-2-oxazoline-4-one (XVI). This was obtained on boiling methyl 3-indolyllactate and N,N'-dimethylguanidine hydroiodide in a NaOEt soln, with a yield of 50%, m.p. 211–211·5° from EtOAc. (Found: C, 64·20; H, 5·23; N, 17·38. $C_{13}H_{13}N_3O_2$ requires: C, 64·18; H, 5·39; N, 17·28%); IR spectrum: 3230, 3100, 1665, 1645 cm^{-1} (Nujol).

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