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Synthesis of 1-*p*-Chlorobenzyl-7-azaindole-3-α-piperidylmethanol as a Potential Antimalarial Agent†

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A single diastereoisomer of 1-*p*-chlorobenzyl-7-azaindole-3-α-piperidylmethanol was found to have antimalarial activity about 0.5 that of quinine when tested in mice against *Plasmodium berghei*. This is the first example of any antimalarial activity in the 7-azaindole class. None of the substituted 7-azaindole intermediates synthesized in this study showed appreciable activity. Also, the low activity of the mixed diastereoisomers indicated that one of the isomers was inactive. An intermediate in the 4-step synthesis, 7-azaindole-3-carboxaldehyde, was prepared in good yield by a Duff reaction, which represents a new and facile method for introducing the 3-formyl group on an N-unsubstituted 7-azaindole.

Very little biological activity has been published for any of the azaindoles other than 3-azaindole (benzimidazole) derivatives.[1] The primary interest in azaindoles has been as potential antimetabolites to naturally occurring indole derivatives. A pharmacological profile of the unsubstituted azaindoles comparing the activities of these compounds with indole, diazaindoles, and purine was reported.[2] Antimalarial activity in plain indoles with antimalarial type and other miscellaneous side chains has not been observed.[3] Before this study none of the reported azaindoles would be considered as candidate antimalarial target compounds. The goal of this study was to explore the potential of 7-azaindole as a new antimalarial nucleus.

The basis for exploring the 7-azaindoles rests on the rationale that the structure of 1-*p*-chlorobenzyl-7-azaindole-3-α-piperidylmethanol is comparable to the quinoline-5-α-piperidylmethanols, and the latter compounds show antimalarial activity. In addition, 7-azaindole having a pK_a = 4.59 and log *P* = 1.82 is isolipophilic with respect to quinoline which has a pK_a = 4.95 and log *P* = 2.03‡

Chemistry. Unsubstituted 7-azaindole is not formylated under normal Vilsmeier reaction conditions and 7-azaindole-3-carboxaldehyde has been made from 7-azagramine.[4,5] A better method to prepare this aldehyde was discovered whereby 7-azaindole is 3-formylated with hexamethylenetetramine in refluxing aq AcOH. The yields in this reaction were consistently good and the product was pure enough for synthetic purposes. Following procedures for the plain indoles[6] 1-alkylation provided 1-*p*-chlorobenzyl-7-azaindole-3-carboxaldehyde plus some quaternary product.[7] The key aldehyde **3** was also prepared by first 1-alkylating[8] and then formylating under Vilsmeier reaction conditions. This experiment confirms earlier observations about Vilsmeier formylation of 7-azaindoles, *i.e.*, that this reaction is facilitated by 1-substitution.[9]

The condensation of **3** with 2-pyridyllithium gave 1-*p*-chlorobenzyl-7-azaindole-3-α-piperidylmethanol as a labile oil which tends to decompose back to its aldehyde and pyridyl components.[10] Low pressure hydrogenation of the side chain pyridyl in its pyridinium salt form provided 1-*p*-chlorobenzyl-7-azaindole-3-α-piperidylmethanol as mixed diastereoisomers. Fractional crystallization separated one of the diastereoisomers as stable microcrystals, and this isomer was tested for antimalarial activity. The second isomer was extremely hygroscopic and apparently inactive as an antimalarial agent.

Several other 3-substituted 7-azaindoles were prepared as possible intermediates with "handles" for the introduction of side chains. These included 3-bromo-7-azaindole,[11] 3-nitro-7-azaindole,[12] and 1-*p*-chlorobenzyl-7-azagramine. Mild MnO_4^- oxidation[13] of the aldehyde **3** gave 1-*p*-chlorobenzyl-3-carboxy-7-azaindole. Only starting material was recovered when the carboxylic acid **9** was treated with 2-PyLi in an attempt to prepare the ketone.[14] *n*-BuLi is known to metalate indoles on the 2 position[15] and the reactive 2-PyLi[16] may have formed a 2-lithio-7-azaindole in our reaction sys-

Table I. Antimalarial Activity and Toxicity of 7-Azaindoles in Mice[a]

Compd	Dose, mg/kg	IMST, days[b]	Toxic deaths[c]
1	640	0.8	0
2	640	0.7	0
3	640	0.5	0
4	640	0.5	0
5	640	0.3	0
6	640	0.3	0
8	10	0.3	0
	40	0.5	0
	160	0.7	0
	320	3.1	0
	640	3.9	2
8a	640	0.5	0
9	640	0.7	0
10	160	0.3	0
	320	0.9	3
	640	–	5
11	640	1.0	0
12	640	0.6	0

[a] Test data supplied by Walter Reed Army Institute of Research. [b] Increase in mean survival time between *Plasmodium berghei* infected mice administered the drug sc and controls. Non-drug-treated mice survived about 6 days following infection with *P. berghei*. Chemical therapy was initiated 3 days postinfection. For details of this test see T. S. Osdene, *et al.*[20] [c] Number of deaths/5 mice.

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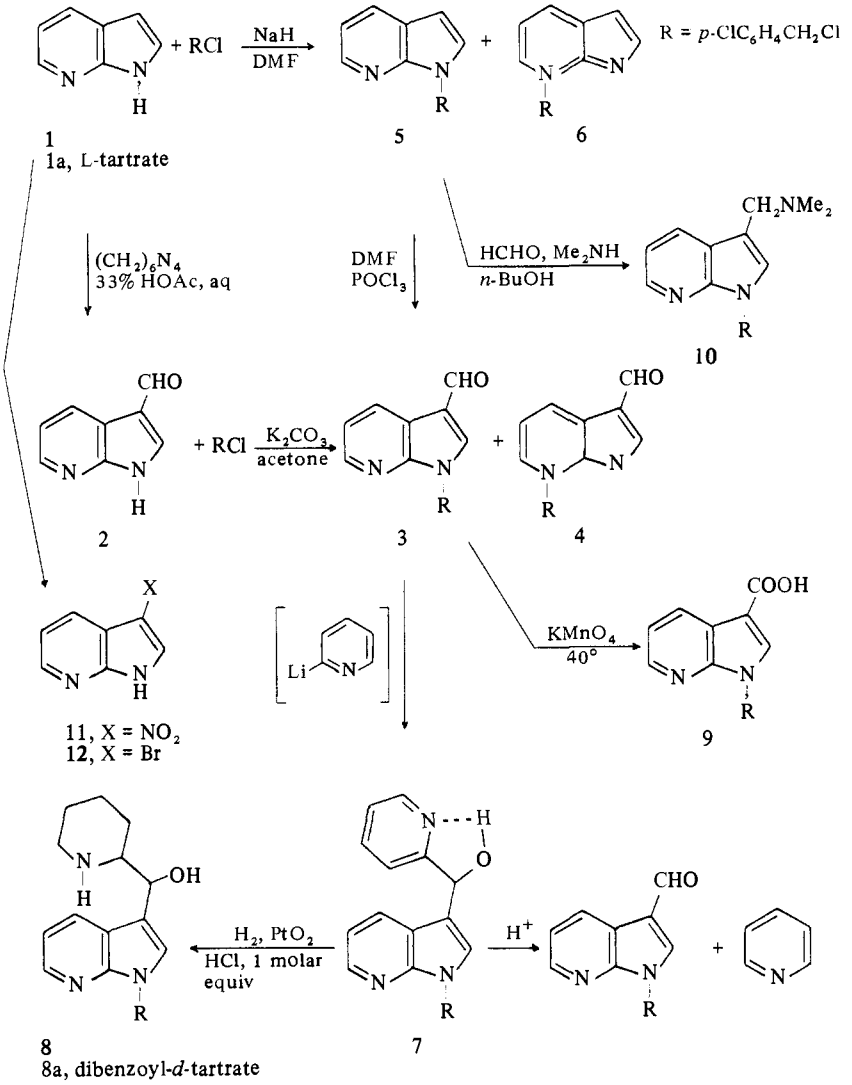
‡The log *P* of 7-azaindole and this comparison with quinoline were provided by Professor Corwin Hansch, whose assistance is gratefully acknowledged.

Scheme I



tem. This would decrease the probability for forming a dianion at the CO_2H function which appears to be an intermediate in the condensation sequence leading to the ketone.§ Also, simple carboxylic acids have failed to form ketones with MeLi.[17] Attempts to prepare 7-azaindole-3-carbinoleamines by treating **3** with dimethylsulfonium methylide to form an oxirane intermediate[18] were not successful.

Biological Results. All of the 7-azaindoles, **1–12**, were tested for antimalarial activity against *Plasmodium berghei* in mice. At 320 mg/kg the single crystalline diastereoisomer of 1-*p*-chlorobenzyl-7-azaindole-3-α-piperidylmethanol (**8**) increased mean survival time by 3.1 days. This is about 0.5 the activity of quinine sulfate which at 160 mg/kg has an IMST of 3.6 days. This is, therefore, the first example of a 7-azaindole derivative with antimalarial activity. The mixed diastereoisomers of this structure were tested as the dibenzoyl-*d*-tartrate salt **8a**, which had an IMST of 0.5 days at 640 mg/kg indicating that the second diastereoisomer is inactive. None of the other 7-azaindoles had an IMST > 1.0 day at 640 mg/kg. Except for **8** and **10** they were also not noticeably toxic at this dose level. This lack of toxicity is notable because several of the other azaindoles are quite toxic. For example, the LD_{50} in mice for 5-azaindole is 16.5 mg/kg, and for 6-azaindole it is 12 mg/kg, compared to 490 mg/kg for 7-azaindole and 316 mg/kg for indole.[2]

§ Private communication from Professor R. E. Lutz.

Compds 1–4, **9**, and **11** were tested *vs. P. gallinaceum* at 120 mg/kg administered sc in chicks and found to be inactive. There were no toxic deaths noted for these 6 compounds.

In the mosquito screen *vs. P. gallinaceum* at a dose level of 0.1%, suppression of oocysts was registered by **4** (75%), **11** (50%), and **12** (50%). Compd **2** (86% deaths) was classified as toxic to the mosquitoes. Compds **1** and **9** were inactive.

Compd **8** has definite CNS effects in mice showing stimulant and possible antidepressant activity.#

Experimental Section**

7-Azaindole was prepd by a known method[19] in an overall yield of 46%. A mono-L-tartrate salt formed and was recrystd from EtOH as white needles, mp 175–177°, which were sol in H_2O. *Anal.* ($C_7H_6N_2 \cdot C_4H_6O_6$) C, H, N

7-Azaindole-3-carboxaldehyde. A soln of 23.6 g (0.20 mole) of 7-azaindole and 42 g (0.30 mole) of hexamethylenetetramine was refluxed with stirring for 6 hr in 250 ml of 33% AcOH (84 g, 1.40 mole, of AcOH and 168 ml of H_2O). The resulting clear yellow soln was dild with 500 ml of H_2O, and the product was allowed to cryst in the refrigerator overnight. Recrystn of the crude product from H_2O gave 14.9 g (50%) of long white needles, mp 216–218° (re-

#These effects were noted by Professor Leo Abood whose assistance is gratefully acknowledged.

**Melting points are corrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical value.

ported[5] 214.5–215°). The product is insol in dil HCl and can be crystd from this solvent. It is sol in dil base. It formed a phenylhydrazone as yellow plates, mp 230–233° dec (reported[5] mp 231–232.5° dec).

The reaction as reported here was run 7 times with variations in reagent and solvent ratios and with quantities of 7-azaindole as small as 0.35 g. Crude yields were consistently in the range of 51–63%. However, a shorter reflux time (3 hr) and gradual addn (30 min) of hexamethylenetetramine lowered the yield to 12.5%.

1-*p*-Chlorobenzyl-7-azaindole. Following known procedures,[8] 6.47 g (0.16 mole) of 57% NaH in oil was washed with petr ether and the last traces of solvent were evapd *in vacuo*. The flask was then cooled in an ice-*n*-PrOH bath and 150 ml of DMF was added, followed by a soln of 17.7 g (0.15 mole) of 7-azaindole in 100 ml of DMF over 15 min with stirring. When the evoln of H_2 stopped, a soln of 24.2 g (0.15 mole) of *p*-chlorobenzyl chloride in 75 ml of DMF was added over 10 min and the mixt was stirred at room temp for 3 hr. Most of the DMF was distd off *in vacuo*. H_2O was added to the residue and the dark oil was extd into Et_2O. A tlc monitor indicated the presence of 2 products. An extn of the Et_2O soln with dil HOAc cleanly removed 7-*p*-chlorobenzyl-7*H*-pyrrolo[2,3-*b*]pyridine. The Et_2O soln contg the desired product was dried (Na_2SO_4), the solvent evapd, and the residue distd *in vacuo* giving 31.1 g of crude yellow oil, bp 157–160° (0.5 mm). The oil crystd from 60–110° petr ether resulting in a 29.7 g (81%) yield of 1-*p*-chlorobenzyl-7-azaindole, mp 35–37°, as long white needles. It tends to decompose on standing for 1 month. It is insol in dil mineral acid but forms an HBr salt, mp 178–181.5°, which oils out in H_2O. The oil can be extd into Et_2O. It seems likely that steric hindrance by the bulky *p*-chlorobenzyl group interferes with salt formation and acid solubility of this compd. *Anal.* ($C_{14}H_{11}N_2Cl$) C, H, N

7-*p*-Chlorobenzyl-7*H*-pyrrolo[2,3-*b*]pyridine. The HOAc ext from the prepn of 1-*p*-chlorobenzyl-7-azaindole was basified to yield 1.82 g of a yellow solid. Crystn from CCl_4-petr ether gave 1.62 g (4.5%) of small yellow needles, mp 120–122°. *Anal.* ($C_{14}H_{11}N_2Cl$) C, H, N

1-*p*-Chlorobenzyl-7-azaindole-3-carboxaldehyde. Method A. To 14 ml of DMF cooled to 0° there was added over 3 min with stirring 6.95 g (0.045 mole) of $POCl_3$. The soln was stirred outside the bath for 10 min and then a soln of 10 g (0.041 mole) of 1-*p*-chlorobenzyl-7-azaindole in 10 ml of DMF was added over 5 min as the temp remained below 20°. The clear soln was warmed at 40–55° for 75 min and then cooled, and 70 g of ice water were added as a solid pptd. A soln of 21.6 g (0.56 mole) of NaOH in 60 ml of H_2O was added and the mixt was brought to boiling for 3 min. On cooling the suspended oil crystd. It was collected, washed well with H_2O, dried, and crystd from CCl_4 giving a 9.94 g (88%) yield of pure white needles, mp 104–106°.

Method B. A mixt of 10 g (0.0685 mole) of 7-azaindole-3-carboxaldehyde, 11.6 g (0.072 mole) of *p*-chlorobenzyl chloride, and 11.3 g (0.082 mole) of K_2CO_3 in 500 ml of Me_2CO was stirred under reflux for 10 hr. After this time a tlc monitor showed that the starting aldehyde was consumed. The Me_2CO was evapd and the residue was taken up in H_2O and EtOAc. The EtOAc phase was extd with 200 ml of 1 *N* HCl in 3 portions to remove 7-*p*-chlorobenzyl-7*H*-pyrrolo[2,3-*b*]pyridine-3-carboxaldehyde as described below. The EtOAc phase was dried (Na_2SO_4), and the solvent was evpd leaving a solid. This was crystd from CCl_4 to yield 15 g (81%) of fine white needles, mp 105–107°. It is insol in acid probably due to both steric hindrance of the *p*-chlorobenzyl substituent and the electron-withdrawing effect of the 3-CHO which lowers basicity of the nucleus. An analytical sample was crystd to mp 107–108°. *Anal.* ($C_{15}H_{11}N_2ClO$) C, H, N

7-*p*-Chlorobenzyl-7*H*-pyrrolo[2,3-*b*]pyridine-3-carboxaldehyde. The HCl extract of the above reaction mixt was made basic to free 3.11 g (16.7%) of a solid. This was crystd twice from EtOH to give 1.82 g of fine yellow needles, mp 142–144°. This product is colorless in aq acid in which it is highly soluble. *Anal.* ($C_{15}H_{11}N_2ClO$) C, H, N

1-*p*-Chlorobenzyl-3-carboxy-7-azaindole. To a soln of 13.6 g (0.050 mole) of 1-*p*-chlorobenzyl-7-azaindole-3-carboxaldehyde in 300 ml of Me_2CO there was added over 15 min with good stirring a soln of 15.8 g (0.10 mole) of $KMnO_4$ in 220 ml of H_2O. During this addn the temp of the reaction mixt was kept at 35–40° using an ice bath. Stirring was then contd for another 45 min outside the bath. The mixt was filtered and the ppt was washed well with 1:1 Me_2CO–H_2O. The solvent was partially removed on a rotary evaporator as an oil appeared, which was extd into Et_2O. The basic aq layer was acidified with HOAc to ppt a solid. This product was crystd from EtOH giving 7.71 g (54%) of tan needles, mp 200–204°. *Anal.* ($C_{15}H_{11}N_2ClO_2$) C, H, N

1-*p*-Chlorobenzyl-7-azagramine Hydrochloride. A soln of 2.43 g (0.010 mole) of 1-*p*-chlorobenzyl-7-azaindole, 0.88 g (0.016 mole) of $Me_2NH_2^+Cl^-$, 0.33 g (0.011 mole) of paraformaldehyde, and 45 ml of *n*-BuOH was refluxed for 1 hr. The solvent was evpd leaving a solid which oiled out upon addn of 50 ml of H_2O. Following the addn of 1 ml of concd HCl the undissolved oil was extd into Et_2O and worked up to give 1.2 g (50% recovery) of 1-*p*-chlorobenzyl-7-azaindole. The aq acidic portion was made basic with K_2CO_3 to free 1.24 g of a second oil which could not be induced to cryst. Addn of 1 equiv of HCl in EtOH to this oil and removal of the solvent provided a cryst salt. Crystn from EtCOMe gave 0.90 g (30%) of fine white needles, mp 202–205° dec. The dihydrochloride was hygroscopic. No attempt was made to improve the yield. *Anal.* ($C_{17}H_{19}N_3Cl_2$) C, H, Cl

1-*p*-Chlorobenzyl-7-azaindole-3-α-pyridylmethanol. A 2-PyLi reagent was generated in the usual manner in 60 ml of Et_2O using 8.9 ml (0.020 mole) of 2.25 *M* BuLi in hexane and 3.16 g (0.020 mole) of 2-bromopyridine.[16] After stirring the reagent at −60° for 30 min a soln of 3.52 g (0.013 mole) of 1-*p*-chlorobenzyl-7-azaindole-3-carboxaldehyde in 20 ml of THF was added over 4 min as the soln became a clear orange. It was stirred for 1 hr at −60°, then allowed to come to room temp during another hour of stirring. The mixt was dild with EtOAc, washed with H_2O, three 50-ml portions of 3% HOAc, Na_2CO_3 soln, and H_2O again, and then dried (Na_2SO_4). A tlc check at this point showed a very strong spot corresponding to about 90% of product plus about 5% of starting aldehyde as the major impurity. Evapn of the solvent gave 5 g of the carbinol as a glass that could not be induced to cryst. The oil was sol in Et_2O, CCl_4, and PhMe but it was insol in *i*-Pr_2O. It was sol in dil HCl where it gradually decompd, apparently back to the aldehyde and pyridine (tlc monitor). The hydrochlorides of the oil were cryst but extremely hygroscopic. An acetate did not cryst. Partial identification was obtd by ir analysis of the carbinol: in $CHCl_3$: 2.94 (OH), 6.22 and 6.66 μ (arom), 6.32, 6.45 μ (7-azaindoles); and the acetate; in $CHCl_3$: 5.74 (acetate), 6.22, 6.67, 6.32, 6.47 μ; tlc, Merck silica gel G, 95:5 PhH–MeOH developer, carbinol R_f 0.43; acetate R_f 0.71.

A critical condn in the prepn of this pyridylcarbinol is that just enough 2-PyLi reagent must be generated to react with the aldehyde function. Excess 2-PyLi appears to react elsewhere in the 7-azaindole nucleus. The pyridylcarbinol was taken on directly to the hydrogenation step.

1-*p*-Chlorobenzyl-7-azaindole-3-α-piperidylmethanol. A soln of 5 g (0.013 mole) of crude 1-*p*-chlorobenzyl-7-azaindole-3-α-pyridylmethanol in 125 ml of EtOH contg 1 molar equiv of HCl was shaken with 300 mg of PtO_2 under H_2 at 2.9 kg/cm² until 3 equiv of H_2 were absorbed. After removing the solvent the resulting product was taken up in 150 ml of H_2O and extd 3 times with EtOAc using NH_4Cl to break an emulsion. The clear light orange aq soln of acid solubles was basified to release an oil, which was extd into EtOAc and dried ($NaSO_4$). A tlc of this soln on Merck silica gel G, 4:1 EtCOMe–MeOH developer, showed 2 spots of about equal intensity at R_f 0.07 and 0.10, corresponding to the two diastereoisomers. After removing the EtOAc from the oil, dry Et_2O was added and the slower moving diastereoisomer eventually crystd from the soln as a powder. It was crystd from PhMe to give 1.14 g (25%) of colorless microcrystals, mp 151–156°. It was homogeneous on tlc using Eastman chromagram 6060 sheets with Me_2CO and also 3:1 Me_2CO–MeOH as the developer. A sample was crystd to mp 154–156° for elemental analysis. *Anal.* ($C_{20}H_{22}N_3ClO$) C, H, N, Cl

The mono- and dihydrochlorides were too hygroscopic to serve as derivs, but a suitable dibenzoyl-*d*-tartrate salt of the mixed diastereoisomers was formed.

1-*p*-Chlorobenzyl-7-azaindole-3-α-piperidylmethanol Dibenzoyl-*d*-tartrate. 1-*p*-Chlorobenzyl-7-azaindole-3-α-pyridylmethanol (6.7 g) was hydrogenated as in the previous expt. This pyridylcarbinol was prepd using a 2.5:1 mole ratio of 2-PyLi to aldehyde and contd several major impurities due to excess reagent. Its purity was about 60%. After hydrogenation and work-up, tlc showed the resulting product contd the double spot corresponding to the two diastereoisomers plus several impurities. Neither diastereoisomer could be induced to cryst from this mixt. Eventually a dibenzoyl-*d*-tartrate salt was formed in Et_2O providing 3.28 g of white powder, mp 130–145°. Crystn attempts were generally unsuccessful but the product seemed to be a reasonably pure mixt of the two diastereoisomers. Tlc on Eastman chromagram sheet 6060 using 3:1 Me_2CO–MeOH developer showed 2 spots at R_f 0.20 and 0.32. *Anal.* ($C_{20}H_{22}N_3ClO \cdot C_{18}H_{14}O_8$) C, 63.90; H, 5.08; N, 5.88. Found: C, 64.11; H, 5.53; N, 5.56.

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Kinetics and Mechanisms of Drug Action on Microorganisms. 13. Comparative Studies on Action of Lincomycin, Clindamycin, and U 24729A against *Escherichia coli*

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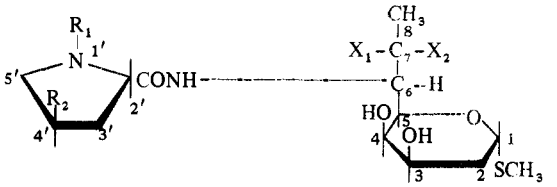
Escherichia coli cultures in broth affected by sub-completely-inhibitory concns of lincomycin (I) exhibit 2 phases of steady-state generation while those affected by the 7(S)-halogenated compounds, clindamycin (II) and U 24729A (III), have only one phase of steady-state generation. The generation rate constants of II- or III-affected cultures have the same functional dependencies on drug concentrations as lincomycin-affected cultures in phase I but are different from those of I in phase II generation. The possibility that lincomycin blocks 2 separate sites in a metabolic sequence whereas the 7(S)-halogenated compds block only the latter in the sequential biochemical process, can rationalize these phenomena. II is 6.6 times and III is 28.5 times as active as I calcd on the basis of molar equivalency. The effects of changes in pH on the activity of I, II, and III against *E. coli* suggest that the unprotonated fraction of the drug concn contributes to the activity. The combined actions of II and III are not antagonistic at any level of activity and can be quantitatively predicted from the separate equivalent dose-response curves of either drug. However, combinations of II or III with I show antagonistic effects which depend on the level of activity. These can be rationalized by the one (*e.g.*, lincomycin) allosterically modifying the receptor site for the other lincosaminide antibiotic.

Lincomycin (I) is an antibiotic produced by *Streptomyces lincolnensis.*[1,2] It has an antibacterial spectrum similar to that of erythromycin and was claimed to be superior[3,4] because of its effectiveness against both erythromycin-susceptible and -resistant strains of Gram-positive coccal organisms. However, it was subsequently found[5,6] that there was a "dissociated type" of cross-resistance in *Staphylococcus aureus* between erythromycin and lincomycin. Lincomycin was therefore modified[7] chemically to serve as a basis for the understanding of structure-activity relations. Thus, analogs of enhanced potency and broadened antibacterial spectrum were prepared.

The main structural effects claimed for *in vitro* activity[7] were (a) the variation of the alkyl substituent at the N′ atom of the pyrrolidine nucleus which changed the antibacterial spectrum of activity; (b) the increase in size of the alkyl group at the C-4′ in the pyrrolidine nucleus which increased lipophilicity and the activity; (c) halogen substitution of the 7-(S) configuration of lincomycin molecule which potentiated antibacterial effects; and (d) the need of



I, lincomycin
$R_1 = CH_3$
$R_2 = C_3H_7$
$X_1 = OH$
$X_2 = H$

II, clindamycin [7(*S*)-chloro-7-deoxylincomycin]
$R_1 = CH_3$
$R_2 = C_3H_7$
$X_1 = H$
$X_2 = Cl$

II. U 24729A [1′-demethyl-4′-depropyl-4′(*R*) and (*S*)-*n*-pentylclindamycin]
$R_1 = H$
$R_2 = C_5H_{11}$
$X_1 = H$
$X_2 = Cl$