Antimicrobial Evaluation of Diastereoisomeric 2-Dimethylaminomethyl-6-phenylcyclohexanols and Related Esters

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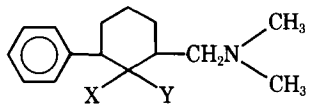
Abstract □ 2-Dimethylaminomethyl-6-phenylcyclohexanone was reduced to give the isomeric 2-dimethylaminomethyl-6-phenylcyclohexanols, which were then converted to a number of novel esters. These derivatives were screened against a wide range of microorganisms. The alcohol possessing an axial hydroxy group had high activity against *Candida albicans*, in contradistinction to the isomer having an equatorial hydroxy function, which was approximately 30 times less active. The esters from the isomeric alcohols demonstrated a low level of antimicrobial activity with the exception of the 10-undecenoyl ester of 2-dimethylaminomethyl-6-phenylcyclohexanol (possessing the equatorial hydroxy group), which showed significant activity against three pathogenic fungi.

Keyphrases □ 2-Dimethylaminomethyl-6-phenylcyclohexanone—conversion to isomeric alcohols and esters, screened for antimicrobial activity □ 2-Dimethylaminomethyl-6-phenylcyclohexanols and esters—screened for antimicrobial activity □ Antimicrobial activity—screening of diastereoisomeric 2-dimethylaminomethyl-6-phenylcyclohexanols and esters

It is well established that β-aminoketones (Mannich bases) have a wide range of chemotherapeutic properties (1–5) including antimicrobial activity (6–10). In addition, reduction of the keto group of Mannich bases has led to compounds with increased pharmacological activity (4). It was decided to prepare the Mannich base from 2-phenylcyclohexanone to compare the activity of this β-aminoketone (I) and related compounds with the corresponding acyclic derivative, 4-dimethylamino-1-phenyl-2-butanone, and its analogous derivatives.

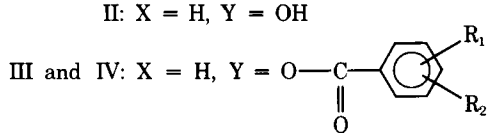
Reduction of I should lead to the formation of isomeric alcohols (II*a* and II*b*), the differences in stereochemistry of which may reflect different potencies against microorganisms. Furthermore, it was hoped to use the concept of latentiation (11) by preparing esters (III and IV) that would be predicted to undergo *in vivo* hydrolysis to the parent alcohols (II*a* and II*b*). The rate of hydrolysis in the series of benzoyl esters would be dependent on several factors, including the Hammett value of the nuclear substituents. In addition, steric impedance of the esters IV containing an axial ester group would be expected to be greater than in the series containing an equatorial ester configuration (III). The rate of hydrolysis of the esters, if carried out enzymatically, also may be dependent on the stereochemical requirements at a receptor site of the enzyme. Thus, a correlation between the rate of release of the alcohols and antimicrobial potency may emerge in this series of compounds.

Finally, it was proposed to synthesize the phenoxyacetyl and 10-undecenoyl esters of II*a* and II*b*, which may lead to the release of phenoxyacetic and 10-undecenoic acids *in vivo;* both of these compounds are known to possess antifungal activity.

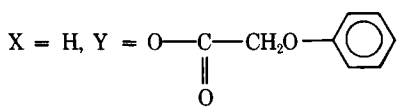


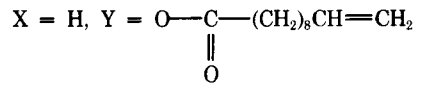
I: X, Y = O

II: X = H, Y = OH



$R_1 = R_2$ = H, Cl, NO_2, OCH_3





EXPERIMENTAL[1]

The preparation of the compounds described in this work was reported previously (12). The compounds in Table I were evaluated using an agar dilution method. In each case a stock solution of the compound was prepared by dissolving 20 mg of the sample in 1 ml of dimethyl sulfoxide, followed by the addition of 3 ml of water. Aliquots of the stock solution were pipetted into melted trypticase soy agar (BBL) to give final concentrations of the compound of 200, 50, 12.5, and 3.1 μg/ml. After hardening, the agar surface was inoculated with appropriately diluted suspensions of the test organisms using a multiple inocula replicating device[2]. The seeded plates were incubated (18 hr at 30° followed by 18 hr at 37°), and the plates were then observed for microbial growth.

The screening techniques for the compounds listed in Table II were as follows. In the antibacterial screen, 1 ml of stock solution (1000 μg/ml) was added to 9 ml of nutrient broth to give an initial concentration of 100 μg/ml and 1:1 dilutions were then added to nutrient broth. All tubes were inoculated with 0.1 ml of a 1:10 dilution of an 18-hr culture of the test organism. All tubes were incubated for 24 hr at 37° and then examined for growth.

The antifungal screen involved the addition of 1 ml of the stock solution (1000 μg/ml) to 9 ml of Sabouraud broth to give an initial concentration of 100 μg/ml and 1:1 dilutions were added to Sabouraud broth. A spore suspension (0.1 ml) of *Trichophyton granulosum* and *Microsporum gypseum* and 0.1 ml of a 1:100 dilution of a broth culture of *Candida albicans* were added to the test tubes containing the various concentrations of the test substances. Tubes containing *C. albicans* were incubated at 37° and tubes containing *M. gypseum* and *Tr. granulosum* were incubated at 28°. Tubes were examined for growth after 4 and 9 days of incubation.

In the antitrichomonal screen, 1 ml of the stock solution (1000 μg/ml) was added to 9 ml of Diamond medium to give an initial

[1] Antimicrobial evaluations were carried out by Dr. Joseph F. Pagano and staff at Smith Kline & French Laboratories, Philadelphia, Pa. (Table I) and by Ayerst Laboratories, Montreal, Quebec, Canada (Table II).

[2] Steers.

Table I—Screening of 2-Dimethylaminomethyl-6-phenylcyclohexanone and Related Alcohol and Esters against Various Microorganisms[a]

Microorganism[b]	I	IIa	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	IIIg
Streptococcus faecalis	>200	>200	>200	200	>200	>200	>200	>200	>200
Staphylococcus aureus (R)[c]	>200	>200	>200	200	>200	>200	>200	>200	>200
Staphylococcus aureus (S)[d]	>200	>200	>200	200	>200	>200	>200	>200	>200
Klebsiella pneumoniae	>200	>200	>200	>200	>200	>200	>200	>200	>200
Pseudomonas aeruginosa	>200	>200	>200	>200	>200	>200	>200	>200	>200
Escherichia coli	>200	>200	>200	>200	>200	>200	>200	>200	>200
Salmonella typhimurium	>200	>200	>200	>200	>200	>200	>200	>200	>200
Trichophyton mentagrophytes	<12	>200	200	200	>200	>200	>200	>200	>200
Mycobacterium smegmatis	50	>200	>200	200	>200	>200	>200	>200	>200
Candida albicans	200	>200	>200	200	>200	>200	>200	>200	>200
Bacillus subtilis	200	>200	>200	50	>200	>200	>200	>200	>200
Fusarium oxysporum	50	>200	>200	200	>200	>200	>200	>200	>200
Penicillium citrinium	50	>200	>200	200	>200	>200	>200	>200	>200
Aspergillus niger	50	>200	>200	200	>200	>200	>200	>200	>200
Cryptococcus neoformans	50	>200	>200	200	>200	>200	>200	>200	>200
Blastomyces dermatididis	>200	>200	>200	200	>200	>200	>200	>200	>200
Xanthomonas vesicatoria	>200	>200	>200	200	>200	>200	>200	>200	>200
Streptococcus pyogenes	>200	>200	>200	50	>200	>200	>200	>200	>200
Sarcina lutea	>200	>200	>200	50	>200	>200	>200	>200	>200

[a] Figures in table are the minimum inhibitory concentrations of the compounds in micrograms per milliliter. [b] The strains of microorganisms in this table are identified by the following numbers: ATCC 9790, SK&F 24390, SK&F 23390, SK&F 4200, SK&F 11320, SK&F 12140, SK&F 11350, SK&F 17410, ATCC 101, SK&F 3470, ATCC 6633, ATCC 9848, ATCC 16040, SK&F 330, EK1, EK2, ATCC 11551, ATCC 8668, and ATCC 9341, respectively. [c] Strain resistant to penicillin G. [d] Strain sensitive to penicillin G.

concentration of 100 μg/ml and 1:1 dilutions were made. Tubes were inoculated with approximately 2.5×10^5 organisms/ml and examined microscopically for growth after 48 hr of incubation.

RESULTS AND DISCUSSION

The preparation and chemical properties of Compounds I–IV were described previously (12). Table I indicates that the β-aminoketone (I) had a good level of activity against the fungus *Trichophyton mentagrophytes* and low levels of activity against seven other microorganisms. Compound I was screened in the plaque inhibition test against rhinoviruses HGP, 28, 1059, and 33342 and was inactive at a concentration of 100 μg. In another screen, I was shown to reduce *Trichinella spiralis* parasites in mice by 24% at a dose of 50 mg/kg (25 mg/kg po and 25 mg/kg sc).

Reduction of I with lithium aluminum hydride gave a 3:1 mixture of isomeric alcohols (IIa and IIb), which was separated by column chromatography. Physicochemical determinations, principally PMR spectroscopy, provided evidence of the stereochemistry of IIa and IIb (Scheme I).

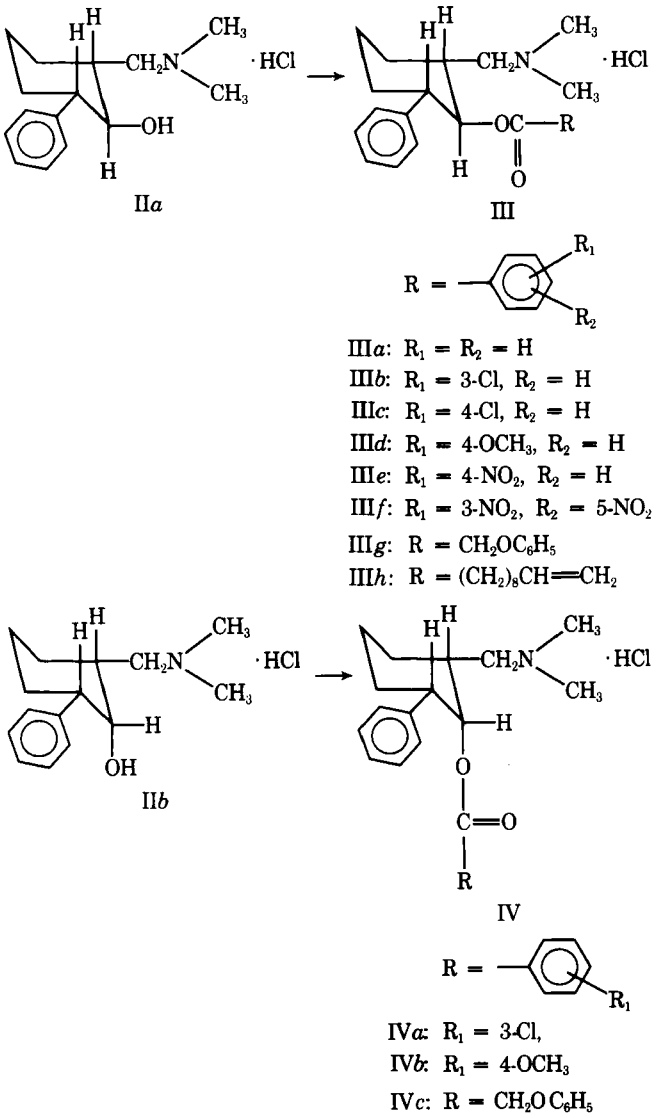
As can be seen from Tables I and II, IIa was virtually devoid of antimicrobial activity. However, alcohol IIb, while failing to show significant activity against most of the microorganisms listed in Table II, showed excellent activity against *C. albicans*. The large potency differences between IIa and IIb against this important pathogenic fungus are noteworthy, illustrating the potency variations between geometrical isomers (13). The lack of activity in IIa may possibly be due to its accessible equatorial hydroxy group, which may allow facile metabolism and detoxification to occur. In addition, the equatorial hydroxy group may form hydrogen bonds readily prior to reaching a site of action. In comparing the bioactivity of I, IIa, and IIb, the broader spectrum of activity of I is noteworthy. It is possible that I, being a Mannich base, can undergo deamination to the corresponding α,β-unsaturated ketone, which may be the active derivative (14). Neither IIa nor IIb would be expected to undergo such a biotransformation.

The masking of the polar hydroxy group by forming the benzoate esters of IIa was accomplished. However, the unsubstituted compound (IIIa) was virtually devoid of antimicrobial activity in the screens chosen. Substitution of the aromatic ester function by electron-withdrawing influences, to increase the predicted *in vivo* hydrolysis, yielded virtually inactive compounds (IIIb, IIIc, IIIe, and IIIf). The ester IIId, containing the electron-repelling methoxy group, would be expected to undergo slower hydrolysis than the other esters, but it failed to demonstrate a significant level of activity. The phenoxyacetyl ester (IIIg) showed virtually no activi-

Table II—Screening of the Isomeric 2-Dimethylamino-6-phenylcyclohexan-1-ols and Related Esters against Various Microorganisms[a]

Microorganism[b]	IIb	IVa	IVb	IVc	IIa	IIIb	IIId	IIIg	IIIh
Candida albicans	3.2	100	100	100	100	100	100	50	6.25[c]
Microsporum gypseum	100	50	25	50	50	100	100	100	3.2
Trichophyton granulosum	100	50	50	100	50	100	100	>100	6.25
Staphylococcus pyogenes (R)[d]	>100	—	—	—	—	>100	>100	>100	100
Staphylococcus pyogenes (S)[e]	>100	—	—	—	—	>100	>100	>100	100
Streptococcus faecalis	100	—	—	—	—	>100	>100	>100	100
Escherichia coli	100	—	—	—	—	>100	>100	>100	100
Aerobacter aerogenes	100	—	—	—	—	>100	>100	>100	100
Salmonella pullorum	100	—	—	—	—	>100	>100	>100	50
Pseudomonas aeruginosa	100	—	—	—	—	>100	>100	>100	100
Proteus mirabilis	100	—	—	—	—	>100	>100	>100	100
Proteus vulgaris	100	—	—	—	—	>100	>100	>100	100
Klebsiella pneumoniae	100	—	—	—	—	100	>100	>100	25
Serratia marcescens	100	—	—	—	—	100	>100	>100	50
Trichomonas vaginalis	100	—	—	—	—	25	100	100	50
Trichomonas foetus	—	50	>100	>100	100				

[a] Figures in table are the minimum inhibitory concentrations of the compounds in micrograms per milliliter. [b] The strains of microorganisms in this table are identified by the following numbers: AY-F-598, AY-F-605, AY-F-604, AY-B-353, AY-B-352, AY-B-355, ATCC 11229, AY-B-357, AY-B-358, AY-B-359, AY-B-360, AY-B-361, ATCC 10081, ATCC 9103, ATCC 30001, and ATCC 30003, respectively. [c] Activity values against resistant strains of *C. albicans* AY-F-610–619 were 20, 16, 32, 62.5, 32, 62.5, 62.5, 32, 32, and 32 μg/ml, respectively. [d] Strain resistant to penicillin G potassium. [e] Strain sensitive to penicillin G potassium.



Scheme I

ty at high doses, suggesting that the ester was not breaking down to yield the established antifungal agent, phenoxyacetic acid.

Attempts to prepare the analogous series of esters from II*b* were not completely successful. Only in the case of the phenoxyacetyl ester (IV*c*) was a crystalline ester obtained uncontaminated with unreacted alcohol. In other cases, a mixture of alcohol and ester was obtained; this mixture proved difficult to separate, although IV*a* and IV*b* were obtained in very low yields. By the time II*b* and its related esters were available for assessment as antimicrobial agents, the screen used to evaluate the previous compounds (Table I) became unavailable. However, the novel esters IV*a*, IV*b*, and IV*c* were evaluated elsewhere and compared to the analogous esters III*b*, III*d*, and III*g* along with the isomeric alcohols II*a* and II*b* (Table II). Esters IV*a*, IV*b*, and IV*c* did not show promising levels of activity, which may indicate failure for hydrolysis to occur under biological conditions.

Finally, attempts to prepare the undecenoyl esters of II*a* and II*b* were undertaken. While an attempt to form the ester from the sterically hindered alcohol II*b* was unsuccessful, II*a* reacted with undecenoyl chloride to give III*h* in good yield. Table II indicates that III*h* possesses high activity against three species of pathogenic fungi. Further studies of III*h* against 10 resistant strains of *C. albicans* showed activity between 16 and 62.5 μg/ml (Table II), considered to be too low a level to warrant further investigation. However, the fact that the undecenoyl ester (III*h*) showed far greater antifungal activity than the corresponding phenoxyacetyl derivative (III*g*) may indicate steric restraints of the aryloxyalkyl ester imposed by the bulky aromatic ring. The olefinic derivative (III*h*) may be able to undergo *in vivo* hydrolysis to undecylenic acid.

In summary, the work demonstrates that another Mannich base (I) shows antimicrobial potency as well as stereospecificity of the isomeric 2-dimethylaminomethyl-6-phenylcyclohexanols (II*a* and II*b*) toward *C. albicans*. Esters of II*a*, with one exception, prepared from II*a* failed to enhance the antimicrobial potency and all of the esters of IV failed to retain the activity against *C. albicans* shown by the precursor alcohol II*b*. The high activity of the undecylenic ester of II*a* may be due to its ability for facile hydrolysis to undecylenic acid, in which case the substituted cyclohexanol can be considered a novel carrier group for the active antifungal agent.

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