Table II—Thimerosal Assay by HPLC after Storage in Trimethoprim–Polymyxin B Eyedrops with Alternatives to Sodium Chloride as Isotonic Agent [a]

Storage Temp.	Control [b] Week 1	Control [b] Week 3	Control [b] Week 6	Boric Acid Week 1	Boric Acid Week 3	Boric Acid Week 6	EDTA Week 1	EDTA Week 3	EDTA Week 6	Propylene Glycol Week 1	Propylene Glycol Week 3	Propylene Glycol Week 6	Glycerol Week 1	Glycerol Week 3	Glycerol Week 6	Mannitol Week 1	Mannitol Week 3	Mannitol Week 6
5°C	NA	NA	100	NA	NA	94	NA	NA	98	NA	NA	103	NA	NA	107	NA	NA	100
25°C	100	103	98	93	91	89	96	86	83	98	97	99	103	100	102	98	100	102
37°C	101	98	94	90	84	72	93	80	64	101	95	91	103	88	84	101	95	94
50°C	98.5	91	85	81	57	35	70	56	43	92	87	81	100	91	82	96	95	84

[a] Initial values at 5°C for each additive were 100%. NA = not assayed. [b] No isotonic agent.

agent. Nonionic compounds, such as these polyhydroxy alcohols, provide suitable alternatives to sodium chloride as isotonic agents in ophthalmic formulations containing thimerosal.

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ACKNOWLEDGMENTS

The author wishes to thank Mr. C. B. Lines for helpful discussions during the preparation of this paper.

Comparative Effects of Selected Phenothiazine Tranquilizers and Antihistaminics on Bacterial Cells and Possible Interactions with Antibiotics

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Received January 13, 1983, from the *College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.* Accepted for publication April 21, 1983.

Abstract □ Evaluation of the antibacterial effect of phenothiazine antihistaminics (trimeprazine, promethazine, and fonazine) and phenothiazine tranquilizers (promazine, chlorpromazine, triflupromazine, and propiomazine) on *Staphylococcus aureus* showed that tranquilizers were more active [minimum inhibitory concentration (MIC) 0.5–1.6 μg/mL] than antihistaminics (MIC > 1.6 μg/mL). The antibacterial activity was found to correlate with both the rate of adsorption of these drugs on the bacterial cells and the surface tension of their solutions. Phenothiazine tranquilizers caused rapid and extensive leakage of potassium ions from bacterial cells, while phenothiazine antihistaminics produced relatively slower leakage of these ions. A study of the effect of the phenothiazines on the antibacterial activity of some antibiotics showed that all phenothiazines produced a synergistic effect with erythromycin and an antagonistic effect with tobramycin. Variable effects were observed with chloramphenicol, and no effect was observed with penicillin. Results were explained on the basis of structural characteristics of the phenothiazines.

Keyphrases □ Phenothiazine tranquilizers—effects on bacterial cells, interaction with antibiotics, *Staphylococcus aureus* □ Phenothiazine antihistaminics—effects on bacterial cells, interaction with antibiotics, *Staphylococcus aureus* □ Antibiotics—interaction with phenothiazine tranquilizers and antihistaminics, *Staphylococcus aureus*

The phenothiazine group encompasses drugs of different therapeutic uses, most important of which are the antihistaminics and the tranquilizers. Most patients receiving these drugs are potential recipients of antibiotic therapy because of their increased susceptibility to infections (1). Synergism and antagonism between antibiotic combinations have been documented. Little attention, however, has been paid to possible interaction between antibiotics and phenothiazines. Some phenothiazines have measurable antibacterial activity *in vitro* (2–4), but are not used clinically for this purpose because of the high doses which would be required. The present work was undertaken to investigate the effect of a variety of combinations of antibiotics and phenothiazines against microorganisms, using *Staphylococcus aureus* as a model.

EXPERIMENTAL

Organism, Drugs, and Antibiotics—*Staphylococcus aureus* NCTC 6571 was used throughout this study. Cultures were maintained on blood agar slants. The drugs used were trimeprazine tartrate[1], fonazine mesylate[1], chlorpromazine hydrochloride[1], promazine hydrochloride[2], propiomazine hydrochloride[2], promethazine[3], and triflupromazine[3]. The antibiotics used were chloramphenicol[4], erythromycin[5], tobramycin[6], and penicillin[6]. Stock solutions of the phenothiazines and the antibiotics were prepared in sterilized distilled water to obtain concentrations of 1.0 mg/mL and 100 μg/mL, respectively.

Determination of Minimum Inhibitory Concentration (MIC)—The MIC values were determined by a standard twofold serial dilution method in brain-heart infusion (BHI) broth[7]. Tubes were inoculated with 10^5 colony forming units (CFU)/mL of *S. aureus*. The lowest concentration showing no visible growth after 18 h of incubation at 37°C was considered as the MIC.

Measurement of Surface Tension—Surface tension was measured by the ring method using a torsion balance[8]. All experiments were carried out in duplicate at 25°C. Distilled water was used as the reference ($\gamma_{25} = 72.4$).

Determination of Potassium Ion Efflux—The test organism was grown at 37°C for 18 h in BHI broth, and the cells were washed with saline to remove extracellular potassium ions before they were suspended in saline. Five milliliters of the suspension was boiled for 30 min, and the amount of potassium

[1] May & Baker, Dagenham, England.
[2] Wyeth, Andover, Mass.
[3] Squibb & Sons, Princeton, N.J.
[4] Parke-Davis & Co., Detroit, Mich.
[5] Abbott Laboratories, North Chicago, Ill.
[6] Eli Lilly & Co., Indianapolis, Ind.
[7] Oxoid Limited, England.
[8] Type OS; White Electronic Instrument Co., Worcestershire, England.

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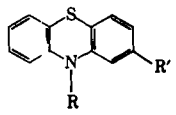
Table I—Comparative Effects of Phenothiazine on *S. aureus*



Phenothiazine	Substituent R	Substituent R′	MIC[a], μg/mL	γ, D/cm	K^+, % Released	Adsorption, % of Control
Tranquilizers						
Chlorpromazine	$(CH_2)_3N(CH_3)_2$	Cl	0.5	56	72	94
Promazine	$(CH_2)_3N(CH_3)_2$	H	0.8	66	62	80
Propiomazine	$CH_2CH(CH_3)N(CH_3)_2$	$COCH_2CH_3$	1.6	61	72	82
Triflupromazine	$(CH_2)_3N(CH_3)_2$	CF_3	0.1	56	74	93
Antihistaminics						
Fonazine	$CH_2CH(CH_3)N(CH_3)_2$	$SO_2N(CH_3)_2$	6.2	63	30	75
Promethazine	$CH_2CH(CH_3)N(CH_3)_2$	H	6.2	65	58	74
Trimeprazine	$CH_2CH(CH_3)CH_2N(CH_3)_2$	H	1.6	61	64	80

[a] Minimum inhibitory concentration.

Table II—Effect of Phenothiazine Drugs on the Antimicrobial Activity of Antibiotic Against *S. aureus*

Phenothiazine (MIC[a], μg/mL)	Antibiotic (MIC[a], μg/mL): Erythromycin (0.12) MIC[a] in Combination	FIC[b]	Tobramycin (0.5) MIC[a] in Combination	FIC[b]	Chloramphenicol (0.12) MIC[a] in Combination	FIC[b]	Penicillin (0.02) MIC[a] in Combination	FIC[b]
Chlorpromazine (0.5)	0.03 + 0.02	0.3	0.5 + 0.1	1.2	0.06 + 0.25	1	0.01 + 0.25	1
Promazine (0.8)	0.05 + 0.1	0.53	0.5 + 0.8	2	0.06 + 0.4	1	0.01 + 0.4	1
Propiomazine (1.6)	0.03 + 0.24	0.4	0.25 + 3.2	2.5	0.06 + 0.8	1	0.01 + 0.8	1
Triflupromazine (0.1)	0.04 + 0.02	0.53	0.25 + 0.1	1.5	0.06 + 0.05	1	0.01 + 0.05	1
Fonazine (6.2)	0.05 + 0.8	0.54	0.5 + 6.2	2	0.03 + 1.5	0.5	0.01 + 3.1	1
Promethazine (6.2)	0.05 + 1.0	0.43	0.5 + 6.2	2	0.03 + 1.5	0.5	0.01 + 3.1	1
Trimeprazine	0.03 + 0.26	0.41	0.25 + 1.6	1.5	0.12 + 0.8	1.5	0.01 + 0.8	1

[a] Minimum inhibitory concentration. [b] Fractional inhibitory concentration.

ions released was determined using a flame photometer[9]. The result was calculated as a percentage of the result obtained for untreated control cells. All experiments were performed in duplicate.

Adsorption and Interaction Experiments—Adsorption was carried out as previously described (5). MIC determinations were performed on BHI broth with an inoculum of 10^5 CFU. Antibiotics and drugs were mixed in checkerboard fashion. Incubation took place at 37°C for 18 h. Synergism was defined as a fractional inhibitory concentration (FIC) index as follows:

$$\text{FIC} = \frac{(A)}{(\text{MIC}_A)} + \frac{(B)}{(\text{MIC}_B)}$$

Where (A) is the MIC of drug A in the presence of drug B, and (MIC_A) is the MIC of drug A alone. (B) and (MIC_B) are defined in the same fashion for drug B. Synergism is present if the FIC index is <1, and antagonism is present if the FIC index is >1. An index value of 1 shows addition (6).

Spectrophotometric Assay—When solutions of the phenothiazines and the antibiotics, separately and in combination at different molecular ratios, were scanned in the UV region[10], no evidence of interaction could be observed in the resulting spectra.

RESULTS AND DISCUSSION

The results (Table I) suggest that there is some relationship between the antibacterial activity of the phenothiazines studied and their surface activity, rate of adsorption, and potassium ion release. Chlorpromazine and triflupromazine, which possess the highest antibacterial activity also showed the highest values for potassium ion release and adsorption and the lowest surface tension. It is possible that by virtue of their high surface activity, these compounds adsorb to the bacterial surfaces, thereby changing the permeability characteristics of the cell membrane. Both drugs have a halogen substitution at R (Cl or CF_3), which increases their lipophilic character and thereby their surface activity. On the other hand, fonazine, which contains the relatively polar *N*-dimethylsulfonyl functional group at R [$SO_2N(CH_3)_2$], causes only a slight potassium ion release and exhibits the least antibacterial activity. The lower antibacterial activity of promethazine and trimeprazine as compared with promazine, all of which have a hydrogen atom at R′, could be attributed to the length of the carbon chain between the two nitrogen atoms in their substituents and also to the branching of their side chain. The relatively higher activity of trimeprazine when compared with promethazine could be due to the additional methylene group at R′ in trimeprazine, which could confer a lipophilic property to the drug.

The antibacterial activity of the phenothiazines and antibiotics, separately and in combination, is shown in Table II. The phenothiazines did not enhance the antibacterial activity of penicillin, and they had an antagonistic effect on the action of tobramycin. The antibacterial activity of erythromycin, on the other hand, was enhanced in the presence of the phenothiazines. Fonazine and promethiazine potentiated the activity of chloramphenicol, while trimeprazine had an antagonistic effect. The remaining phenothiazines did not affect the antibacterial activity of chloramphenicol. The variable results obtained in these experiments are in agreement with previously published reports (7).

The potassium ion efflux from bacterial cells after exposure to the phenothiazines could be due to the effect of these drugs on the bacterial cell membrane. The same type of effect could facilitate the entry of an antibiotic into the bacterial cell and thus potentiate the activity of that antibiotic. The antagonistic effects observed with some phenothiazine-antibiotic combinations might be explained on the basis that these drugs could unselectively block certain receptor sites essential to the action of the antibiotics.

The present results suggest that the action of phenothiazines on the bacterial membrane cannot be the sole factor that determines the type and degree of interaction with antibiotics. Since spectrophotometric measurements of mixtures of antibiotics and phenothiazines excluded chemical interaction, there must be another site of activity of the phenothiazines in the bacterial cell. This type of interaction may have clinical implications in psychotic patients who have to take antibiotics while on phenothiazine tranquilizers. Further work, using different antibiotics in combination with these drugs against different organisms, as well as *in vivo* studies are needed to investigate such interactions.

[9] EEL Flame Photometer, Model 150.

[10] Pye Unicam SP 8100 recording spectrophotometer.

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ACKNOWLEDGMENTS

Presented at the 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 1981.

The authors are grateful to the companies for the courtesy of supplying drugs and antibiotics. Thanks are also given to Mr. H. El-Sammani for his technical assistance and to Mr. K. Abbas for typing this manuscript.

Structural Determination of Nootkatol, a New Sesquiterpene Isolated from *Alpinia oxyphylla* Miquel Possessing Calcium-Antagonistic Activity

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Received February 24, 1983, from the **Faculty of Pharmacy, Tokushima-Bunri University, Tokushima-shi, Tokushima 770*, the ‡*Suntory Institute for Bioorganic Research, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618*, and the §*Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, Japan.* Accepted for publication April 28, 1983.

Abstract □ Nootkatol, a new sesquiterpene possessing calcium-antagonistic activity, was isolated from *Alpinia oxyphylla* Miquel and characterized as (2*R*,4*R*,5*S*,7*R*)-eremophil-1(10),11-dien-2-ol.

Keyphrases □ Nootkatol—isolation from *Alpinia oxyphylla* Miquel, calcium-antagonistic activity □ Calcium antagonists—isolation of nootkatol from *Alpinia oxyphylla* Miquel

In the course of our survey of various crude drugs prepared from plant materials for pharmacologically active metabolites, we have found that a methanolic extract of the fruit of *Alpinia oxyphylla* Miquel (Zingiberaceae) has calcium-antagonistic activity in the rabbit aorta. Other work (1–3) on calcium antagonists, such as verapamil and gallopamil, have revealed that such drugs selectively inhibit the potassium chloride-induced contraction of vascular smooth muscle. In this paper, we have isolated for the first time a substance possessing calcium-antagonistic activity from a plant source.

EXPERIMENTAL[1]

Method of the Bioassay—Rabbits (2–3 kg) were killed by cervical dislocation. The aortas (60 × 4 mm) were removed and cut into helical strips. The strips were mounted vertically in a 20-mL organ bath containing Krebs-Ringer bicarbonate solution of the following composition (mM concentration): NaCl, 120; KCl, 4.8; $CaCl_2$, 1.2; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 25.2; and glucose, 5.8, pH 7.4. This was bubbled with a gas mixture of oxygen-carbon dioxide (95:5) and maintained at 37°C. A resting tension of 1 g was applied to the strips and tension changes were isometrically recorded with a force-displacement transducer.

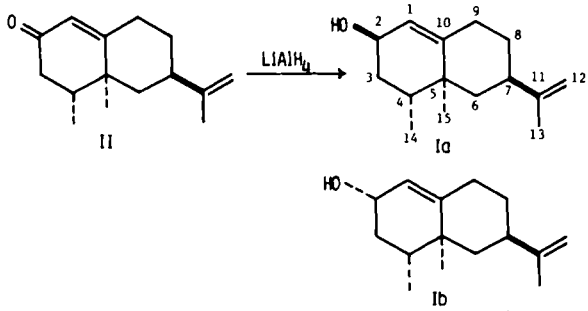
Isolation—The dried, powdered fruit[2] (10 kg) of *A. oxyphylla* Miquel were extracted three times with cold methanol (20 L). The methanolic extract was concentrated *in vacuo*, and the residue (1 kg) was partitioned between ethyl acetate and water. The aqueous layer was extracted with 1-butanol. The pharmacologically active ethyl acetate fraction (605 g) was chromatographed on silica gel[3] (benzene–ethyl acetate). The fractions containing the bioactive material [benzene–ethyl acetate (9:1)] were combined and further purified on a silica gel column with *n*-hexane–acetone (97:3) to afford 3.0 g of an active substance (Ia, 0.03% yield), named nootkatol, as colorless needles, mp 78–80°C (recrystallized from *n*-hexane), $[\alpha]_D$ + 208° (*c* 1.1 $CHCl_3$); IR (KBr): 3250 cm^{-1} (OH); MS: *m/z* 220 (M^+, 53%) and 177 (100); ^{1}H-NMR ($CDCl_3$): δ 0.89 (s, 3, C(5)—CH_3), 0.89 (d, 3, *J* = 6 Hz, C(4)—CH_3), 1.01 (t, 1, *J* = 13 Hz, C(6)—βH), 1.24 (dq, 1, *J* = 12 Hz and *J* = 4 Hz, C(8)—βH), 1.55 (m, 1, C(3)—βH), 1.63 (dt, 1, *J* = 13 Hz and *J* = 4 Hz, C(3)—αH), 1.71 (m, 3, C(11)—CH_3), 1.71 (m, 1, C(4)—βH), 1.79 (m, 1, C(8)—αH), 1.89 (dt, 1, *J* = 13 Hz and *J* = 3 Hz, C(6)—αH), 2.13 (m, 1, C(9)—βH), 2.23 (tt, 1, *J* = 13 Hz and *J* = 3 Hz, C(7)—H), 2.32 (m, 1, C(9)—αH), 4.06 (m, 1, C(2)—H), 4.68 (m, 2, C(12)—H_2), and 5.49 ppm (bd, 1, *J* = 5 Hz, C(1)—H); ^{13}C-NMR ($CDCl_3$): δ 15.21 (q, C-14), 16.83 (q, C-15), 20.79 (d, C-4), 32.54 (t, C-6*), 32.62 (t, C-8*), 34.98 (q, C-13), 36.21 (t, C-3), 38.32 (s, C-5), 40.72 (d, C-7), 44.60 (t, C-9), 64.37 (d, C-2), 108.71 (t, C-12), 121.73 (d, C-1), 148.55 (s, C-11), and 150.13 ppm (s, C-10). Assignments are interchangeable between carbon atoms with asterisks.

Anal.—Calc. for $C_{15}H_{24}O$: C, 81.76; H, 10.98. Found: C, 81.53; H, 11.28.

Reduction of Nootkatone (II)—To the ether solution (50 mL) of nootkatone[4] (1.9 g), 200 mg of $LiAlH_4$ was added and stirred overnight at room temperature. The suspension was treated as usual and extracted with ether. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel [*n*-hexane–acetone (97:3)]. Nootkatol (Ia) (40 mg) was eluted, followed by epinootkatol (Ib) (oily, 1980 mg). The aforementioned synthetic nootkatol was identical by IR, optical rotation, and melting point comparison with the material isolated from *A. oxyphylla*.

RESULTS AND DISCUSSION

From the fruit of *A. oxyphylla* Miquel, a calcium-antagonistic substance, named nootkatol, was isolated. In the mass spectrum the molecular ion (*m/z* 220) indicates a molecular formula of $C_{15}H_{24}O$. The single oxygen func-



Scheme I—Reduction of nootkatone (II) to nootkatol (Ia).

[1] Melting points were obtained on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotation was recorded on a Jasco DIP-180 digital polarimeter. IR spectra were obtained on a Shimadzu IR-27G photometer. ^{1}H-NMR spectra were recorded on a Nicolet NT-360 spectrometer. ^{13}C-NMR spectra were recorded on a Hitachi R-22 spectrometer. Mass spectra were obtained on a Shimadzu LKB-9000B.

[2] Purchased from Nippon Hunmatsu Yakuhin, Ltd., Osaka, Japan.

[3] Silica gel 60 (230–400 mesh); Merck.

[4] Supplied by Shiono Kohryo Co., Ltd., Osaka, Japan.

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