Anal.—Calcd. for $C_{20}H_{20}ClN_3O \cdot 2HCl$: C, 56.28; H, 5.20; Cl (ionic), 16.62. Found: C, 55.95; H, 5.36; Cl, 16.26.

4-(6-Chloro-2-methoxy-9-acridylamino)-α-pyrrolidino-o-cresol Dihydrochloride Sesquihydrate.—One-third of the residue from the preparation of II was treated as in that experiment except that 27.8 Gm. (0.1 mole) of 6,9-dichloro-2-methoxyacridine replaced 4,7-dichloroquinoline. The hot reaction mixture was filtered in order to remove insoluble 6-chloro-9-(4-hydroxyanilino)-2-methoxyacridine. Cooling the filtrate gave 30 Gm. (56% yield) of dihydrochloride, m.p. 245–249° dec. It was recrystallized from alcohol-acetone, m.p. 252–253° dec.

Anal.—Calcd. for $C_{25}H_{24}ClN_3O_2 \cdot 2HCl \cdot 1^1/_2H_2O$: C, 56.25; H, 5.47. Found: C, 56.54; H, 5.37.

p-Acetamido-α-hexamethylenimino-o-cresol Monohydrochloride.—A mixture of 15.1 Gm. (0.1 mole) of *p*-acetamidophenol, 9.9 Gm. (0.1 mole) of hexamethylenimine (5), 3 Gm. (0.1 mole) of paraformaldehyde, and 25 ml. of alcohol was heated to boiling for 4 hours. When cooling gave no precipitate, solvent was removed by distillation under reduced pressure. The residual dark oil was dissolved in ether, and excess alcoholic hydrogen chloride added to precipitate a yellow viscous oil. After decantation of the ether, solution in hot isopropyl alcohol and cooling gave 15.3 Gm. (51% yield) of product, m.p. 192–194°. Recrystallization from the same solvent elevated the melting point to 194.5 to 195°.

Anal.—Calcd. for $C_{15}H_{22}N_2O_2 \cdot HCl$: C, 60.29; H, 7.76. Found: C, 60.46; H, 7.96.

4-(7-Chloro-4-quinolylamino)-α-hexamethylenimino-o-cresol (IV).—A mixture of 14.3 Gm. (0.0478 mole) of *p*-acetamido-α-hexamethyleniminо-*o*-cresol monohydrochloride and 20 ml. of 20% hydrochloric acid was heated at reflux temperature for 1 hour. Sodium hydroxide solution was added to the cooled solution until it was barely acidic to Congo red. After the addition of 9.5 Gm. (0.048 mole) of 4,7-dichloroquinoline, the mixture was heated on a steam bath for 2 hours. Then, with cooling, it was made basic to litmus with 10% sodium hydroxide. The precipitated yellow solid was recrystallized twice from benzene to give 3.8 Gm. (21% yield) of IV, m.p. 212–214° dec.

Anal.—Calcd. for $C_{22}H_{24}ClN_3O$: C, 69.19; H, 6.33. Found: C, 69.31; H, 6.35.

It was found that following the procedure for II, which does not entail isolation of a purified acetamido Mannich phenolic base, actually led to a better yield of IV (45%).

4-(7-Chloro-4-quinolylamino)-α-(4-methyl-1-piperazinyl)-o-cresol (V) Trihydrochloride Dihydrate.—Procedure for II was followed; however, the free base was extracted with chloroform instead of being isolated. The extract was washed with water, dried over potassium carbonate, and treated with excess alcoholic hydrogen chloride. The addition of acetone completed precipitation of product. Recrystallization from alcohol gave 28 Gm. (53% yield) of bright yellow crystalline powder, m.p. 240–300° dec. It is very soluble in water.

Anal.—Calcd. for $C_{21}H_{23}ClN_4O \cdot 3HCl \cdot 2H_2O$: C, 47.73; H, 5.72. Found: C, 47.67; H, 5.93.

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Antifungal Properties of Perfume Oils

By JASPER C. MARUZZELLA

The antifungal properties of 30 perfume oils were tested by allowing the organisms to grow in varying concentrations of the oils *in vitro*. Fifteen of the perfume oils inhibited the growth of all test organisms at concentrations ranging from 1:500 to 1:13,000. The remaining 15 oils inhibited some of the test organisms at concentrations up to 1:11,000. Oil of rose no. 81412 otto type, crab apple blossom, and rose briar were found to possess marked antifungal properties. The dermatophytes were extremely susceptible to many of the perfume oils at minute concentrations.

PERFUME OILS were demonstrated to possess remarkable fungicidal properties when studied by the filter paper disk method (1). However, the oils were tested in the undiluted form at which strength they are rarely used in cosmetics and medicaments. Small amounts of perfumery materials are added to toilet articles and dermatological products in an attempt to render the item more fragrant and to mask unpleasant odors. Whether such minute concentrations possess germicidal properties has been suggested (2) but not experimentally established. This investigation was undertaken in order to determine the mimimal concentration of perfume oil needed to inhibit the growth of fungi *in vitro*.

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TABLE I.—INHIBITORY CONCENTRATIONS OF PERFUME OILS ON FUNGI

Oils	Test Organisms					
	A. niger	*S. schenkii*	*P. ovale*	*C. albicans*	*M. gypseum*	*T. mentagropyhtes*
Arabian N	1:1000	1:500	1:1000	1:1000	1:7000	1:6000
Ashton villa no. 6	1:1000	1:1000	0[a]	1:1000	1:6000	1:7000
Bluebell bouquet	1:1000	1:2000	1:500	1:1000	1:3000	1:6000
Bluestone bouquet	1:1000	1:1000	1:1000	1:1000	1:3000	1:4000
Bouquet no. 22	1:500	1:3000	0	0	1:13,000	1:13,000
Bouquet no. 821 lemon odor	1:500	1:1000	1:500	1:500	1:1000	1:3000
Carylopsis no. 602	1:1000	1:1000	0	1:500	1:3000	1:4000
Chypre french type	1:500	1:3000	0	1:500	1:7000	1:11,000
Cologne American	1:500	1:500	0	0	1:3000	1:3000
Cologne "F" European	1:500	1:500	0	1:500	1:1000	1:2000
Colonial bouquet	1:500	1:1000	1:500	1:1000	1:3000	1:6000
Crab apple blossom	1:2000	1:2000	1:1000	1:1000	1:7000	1:7000
Eau de quinine	1:500	1:1000	0	1:500	1:1000	1:1000
Elder buds	1:500	1:1000	0	1:500	1:2000	1:2000
Gardenia, JM	1:1000	1:1000	1:500	1:1000	1:3000	1:6000
Geranium bouquet	1:1000	1:1000	1:500	1:1000	1:2000	1:1000
Jasmine ordinary	1:500	1:1000	0	0	1:6000	1:4000
Jockey club	1:500	1:1000	0	1:500	1:1000	1:4000
Lilas blanc	1:1000	1:2000	1:500	1:500	1:6000	1:7000
Lilas blanc, L. S.	0	1:500	0	0	1:1000	1:500
Lilac water	1:1000	1:1000	1:500	1:1000	1:4000	1:5000
Neutralizer F.A.	1:1000	1:1000	0	0	1:4000	1:4000
Oriental bouquet no. 225	1:1000	1:2000	1:500	1:2000	1:5000	1:5000
Osheana	1:1000	1:3000	0	1:500	1:7000	1:7000
Palma bouquet	1:1000	1:1000	0	1:500	1:3000	1:3000
Pine bouquet supreme	0	0	0	0	1:1000	1:2000
Rose briar	1:2000	1:2000	1:1000	1:1000	1:6000	1:6000
Rose no. 81412 otto type	1:5000	1:11,000	1:1000	1:3000	1:13,000	1:10,000
Rose odorata	1:1000	1:4000	1:1000	1:1000	1:3000	1:6000
Sweetgrass	1:1000	1:1000	1:1000	1:1000	1:3000	1:2000

[a] No inhibition at 1:500 concentration.

MATERIALS AND METHODS

The antifungal properties of 30 perfume oils were determined against growing cultures of *Aspergillus niger* ATCC 6277, *Sporotrichum schenkii* CDC 629, *Pityrosporum ovale* ATCC 12078, *Candida albicans* ATCC 10231, *Microsporum gypseum* CDC A-352, and *Trichophyton mentagrophytes* ATCC 9533. All of the test organisms were cultivated in Sabouraud maltose broth at 22 to 24° and subcultured every 7 days.

Stock solutions of each perfume oil were prepared in 95% ethyl alcohol. Aliquots of the stock solution were added to melted Sabouraud maltose agar, the contents were hand shaken and poured into Petri dishes. One-hundred milliliters of 7-day broth cultures was hand shaken thoroughly to mix spores and mycelial fragments. One-half milliliter of this fungal suspension was used as the inoculum for each dish and 5 ml. was used to inoculate 100-ml. broths used in subsequent experiments. Inhibitory levels were recorded at the end of 4 days at 22 to 24°. All perfume oils were tested in triplicate. Controls were included to demonstrate the lack of inhibition by the alcohol solvent employed. Replicate tests were conducted on different days in an attempt to minimize the day-to-day variation in the test procedure.

RESULTS AND DISCUSSION

The values listed in Table I are the minimal concentrations of oil found to inhibit growth of the test organisms. It may be observed that 15 of the 30 perfume oils produced inhibition of all test organisms at concentrations varying from 1:500 to 1:13,000. The remaining 15 oils prevented the growth of some of the test organisms at concentrations from 1:500 to 1:11,000. Perfume oil of lilas blanc L.S. and pine bouquet supreme showed slight antifungal properties while rose no. 81412 otto type, crab apple blossom, and rose briar were found to possess inhibitory action at high dilutions. Further inspection of the data in Table I seems to indicate that *T. mentagrophytes* and *M. gypseum* were extremely sensitive to all of the oils while *P. ovale* was resistant.

The data presented would tend to support the view that small amounts of certain perfume oils might be used effectively in the control of some dermatophytes. The concentrations used in this study fall well within the limits of their use in scenting toilet articles and medicaments.

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