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# ANTIFUNGAL ACTIVITIES OF BIS*ISO*QUINOLINIUM AND BISQUINOLINIUM SALTS

BY

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We have previously reported (Collier, Potter, and Taylor, 1953) that certain polymethylene bis*iso*quinolinium salts powerfully inhibit bacterial growth *in vitro*. In spite of the relatively high subcutaneous toxicity of these compounds in mice it seemed possible that they might safely be applied to skin. They were therefore examined against fungi which infect keratinous structures of man and animals for inhibitory activity, which some of them proved to possess in high degree. Consequently further members of the bis*iso*-quinolinium series and some corresponding bisquinolinium salts were prepared and their antifungal and toxic properties explored.

## METHODS

*Compounds.*—The compounds are described in detail in the chemical section. In the biological section they are described by abbreviations corresponding to those adopted by Barlow and Ing (1948). Thus tetradecamethylene bis*iso*quinolinium is abbreviated to BIQ 14 and the corresponding quinolinium to BQ 14. Generally the quaternary iodides were used; but to obtain more concentrated solutions the methosulphates of BIQ 14 and BIQ 16 were employed.

*Antifungal Tests.—In vitro* antifungal properties were investigated in 11 strains of the 6 species named in Table I according to the terminology adopted by Conant, Smith, Baker, Callaway, and Martin (1954). Seven of the strains used had been isolated from man and the remainder from domestic animals. In all experiments, except those comparing the sensitiveness of different strains or species to drugs, we used a single strain of *Trichophyton mentagrophytes* that had been isolated from man.

*Fungistatic Activity.*—Tests of growth inhibition were carried out in Sabouraud's broth, composed of 1% peptone ("Eupeptone" No. 2, A. & H.) and 4% glucose in water. Drugs were serially diluted by 1 in 2 in 2 ml. volumes of broth and autoclaved at 10 lb./sq. in. for 10 min. The dilutions were inoculated with a ground suspension of fungal culture in Sabouraud's broth. After inoculation, tubes were incubated at 27° C. and read by eye after 3 days and subsequently. End-points were expressed as the minimal inhibitory concentration (MIC) and final results as the geometric mean MIC of several figures obtained independently.

Several materials were examined for ability to antagonize the growth inhibition exerted by BIQ 14 or BIQ 16. In addition to those listed in Table II, bovine hair and serum, dog hair and rabbit serum were examined. The materials were included in the media before autoclaving, except sera and lecithins, which were added aseptically to sterilized media.

*Fungicidal Activity.*—In tests of fungicidal activity a suspension containing about 5,000,000 spores/ml. saline was prepared from a 7–10 day culture of *T. mentagrophytes*. Tubes containing 2 ml. volumes of serially diluted drug were inoculated with 0.04 ml. spore suspension and incubated. After a measured time each tube was then shaken and one loopful (4 mm. diameter) transferred into 4 ml. 2% dried bovine bile ("Bacto-Oxgall") in Sabouraud's broth. The bile inhibited the antifungal action of any drug transferred with the inoculum. These subcultures were examined for fungal growth after 14 days' incubation at 27° C. Two series of fungicidal tests were carried out. In one, spore suspensions were incubated in concentrated solutions of drug in saline for periods between 10 min. and 24 hr. at 20° C., phenol being used for reference. In the other series, spore suspensions were incubated in drug solutions in Sabouraud's broth at 27° C. for up to 7 days.

*Toxicity Tests.*—In acute subcutaneous and oral toxicity tests in mice, drugs were administered in aqueous solution. The method of Miller and Tainter (1944) was used to estimate LD50 and its standard error. Tests of intradermal toxicity in rabbits were carried out by injecting 0.04 ml. of solutions of drug in saline into the shaved skin. In all acute toxicity tests the effects of drugs were observed for 7 days after treatment.

In subacute toxicity tests in rabbits, drugs were administered once daily, except on Saturdays and

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Sundays. Saline solutions of drugs were instilled into one of each pair of eyes; or lotions in 50% alcohol were applied to the shaved skin. These lotions were applied also to the unbroken skin of the forearm of 3 human volunteers daily for 4 weeks.

## RESULTS

### *Antifungal Activities*

*Effect of Chain-length and End-group.*—The relationship between length of polymethylene chain in the BIQ series and ability to inhibit growth of a strain of *Trichophyton mentagrophytes* is expressed in Fig. 1. There is a fairly sharp rise in potency as chain-length increases from 10 to 14 methylene groups. With further increase in chain-length, potency at 3 days falls gradually; but at 14 days differences between most of the chain-lengths have vanished, giving a plateau of higher activity extending from BIQ 14 to BIQ 19. The bisquinolinium compounds were almost equiactive to the corresponding bis*iso*quinolinium against the same strain of *T. mentagrophytes*, the effect of altering chain-length being therefore strictly comparable. For example, in 5 experiments with this fungal strain in which their activities were compared directly, the MICs of BIQ 14 iodide were 1.89 μg./ml. at 3 days and 5.74 μg. at 14 days, and of BQ 14 iodide 1.89 and 3.79 μg. respectively. In comparable tests with other drugs, the MICs in μg./ml. at 3 and 14 days were: dibromopropamidine isethionate, 160 and 640; undecylenic acid, 40 and 80; cetrimide, 12.5 and 25.

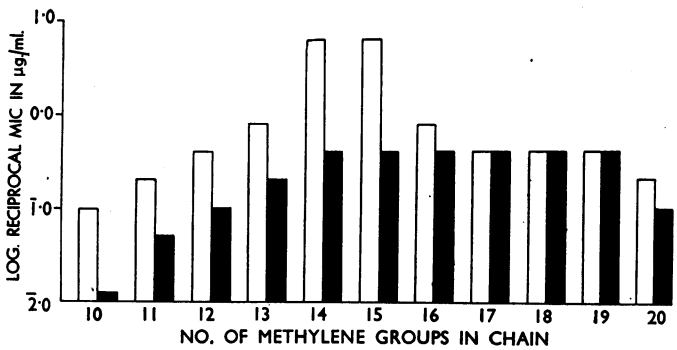


FIG. 1.—Relation of number of methylene groups in chain of bis*iso*quinolinium compounds to activity against *T. mentagrophytes* in Sabouraud's broth at 27° C. White, after 3 days' incubation; black, after 14 days'.

*Action Against Other Strains and Species.*—Results obtained with the one strain of *T. mentagrophytes* justified further investigations of the more active compounds. For this, BIQ 14, BIQ 16, BQ 14, and BQ 16 were selected for reasons discussed below. The results of *in vitro* tests of the activities of these compounds against a number of strains and species of fungi that attack skin are summarized in Table I, from which it will be seen that all four potently inhibited all strains of fungi studied. The compounds showed approximately equal activities, except against *Candida albicans*, which was more sensitive to the hexadecamethylene chain-length. Again for reasons discussed below, BIQ 16 was selected for further study.

*Antagonism of Antifungal Activity.*—Table II shows that sera and hair exerted a slight antagonistic action upon the effect of BIQ 16 on *T. mentagrophytes*. A comparable effect was also seen when the conditions of the experiment were varied, in respect of either the serum employed (bovine, rabbit), the hair (bovine, dog), the inhibitory compound (BIQ 14) or the species of fungus (*T. verrucosum*). Table II also shows that bovine bile was the most potent antagonist: this was used in tests of fungicidal activity.

TABLE I

ACTIVITIES OF BIQ 14, BIQ 16, BQ 14 AND BQ 16 IODIDES AGAINST SOME FUNGI INFECTING SKIN

Minimal inhibitory concn. (μg./ml.) after 14 days' incubation in Sabouraud's broth at 27° C. In parentheses, number of strains examined.

| Species | BIQ 14 | BIQ 16 | BQ 14 | BQ 16 |
|---|---|---|---|---|
| *Candida albicans* .. .. | 5·0 | 1·25 | 7·0 | 1·8 |
| *Microsporum Audouini* .. | 1·25 | 0·625 | 1·25 | 0·44 |
| *Microsporum canis* (2) .. | 1·25–5 | 1·25 | 1·25–2·5 | 1·25 |
| *Trichophyton mentagrophytes* (5) | 1·25–10 | 0·625–5 | 0·625–5 | 0·3125–5 |
| *Trichophyton rubrum* .. .. | 2·6 | 2·5 | 1·8 | 1·8 |
| *Trichophyton verrucosum* .. | 0·44 | 0·44 | 0·44 | 0·3125 |

TABLE II

EFFECT OF VARIOUS MATERIALS ON THE INHIBITION OF GROWTH OF *T. MENTAGROPHYTES* BY BIQ 16 METHOSULPHATE IN SABOURAUD'S BROTH AT 27° C.

| Material added to Medium | | MIC (μg./ml.) at 14 days |
|---|---|---|
| Nature | % Concn. | |
| Thymine | 0·1 | 1·25 |
| Uracil | 0·1 | 2·5 |
| Human hair | — | 10 |
| Human serum | 10 | 5 |
| Horse serum | 10 | 10 |
| "Tween 80" | 0·5 | 10 |
| "Lubrol" | 0·5 | 20 |
| Egg lecithin | 0·1 | 80 |
| Vegetable lecithin | 0·1 | 125 |
| Dried bovine bile | 2 | 4,000 |
| None | | 1·25 |

*Fungicidal Action.*—After exposing spores of *T. mentagrophytes* to 25 mg. phenol/ml. saline at 20° C. for 10 min., viable spores could not be detected on subculture. On the other hand, in similar conditions, spores remained viable after exposure to 50 mg. BIQ 16 methosulphate/ml. saline for 24 hr. The fungicidal action of BIQ 16, however, was readily demonstrated when spore suspensions were incubated in broth at 27° C., as will be seen from the experiment illustrated in Table III. Subcultures were negative after 7 days' incubation of spores in 1.25 μg./ml. of BIQ 16 methosulphate, which is approximately the MIC. From this and comparable experiments with BIQ 14, it was concluded that these compounds are highly fungicidal when acting in nutrient medium for several days.

TABLE III

FUNGICIDAL ACTION AGAINST *T. MENTAGROPHYTES* OF BIQ 16 METHOSULPHATE AT 27° C.

| Time of Incubation of Spore Suspension | Minimal Fungicidal Concn. (μg./ml.)* |
|---|---|
| 5 min. | >640 |
| 1 hr. | >640 |
| 1 day | 160 |
| 2 days | 80 |
| 7 days | 1·25 |

* Lowest concn. at which subcultures in 2% bile broth failed to grow.

### *Toxicity*

*Acute.*—In mice the acute subcutaneous LD50 values (mg./kg.) for the iodides were:

BIQ 14, $24 \pm 1.1$; BIQ 16, $29 \pm 1.6$; BQ 14, $16 \pm 2.3$; and BQ 16, $18 \pm 0.5$. The acute oral LD50 values (mg./kg.) for the methosulphates were: BIQ 14, $228 \pm 13$; BIQ 16, $275 \pm 19$.

In a series of intradermal injections in rabbits, a solution containing 80 μg. BIQ 14 iodide/ml. saline caused erythema followed by necrosis and healing in 4 of 9 injection sites and a similar solution of BIQ 16 caused similar reactions in 5 of 9 sites. When the solutions of either compound were diluted to half this concentration, reactions were observed in none of 9 sites with BIQ 14 and in 1 of 9 with BIQ 16.

*Subacute.*—Solutions containing 1 mg. BIQ 14 or BIQ 16 methosulphate/ml. saline appeared to be without effect on the eyes of rabbits when instilled daily over a period of two weeks.

When lotions containing 5 or 2.5 mg. BIQ 16 methosulphate/ml. 50% aqueous alcohol were applied daily to the shaved skins of rabbits over a period of 4 weeks, no reaction to the drug was seen, although the alcoholic medium caused slight temporary flushing. On the other hand a lotion of 5 mg. BIQ 14 methosulphate/ml. aqueous alcohol caused marked erythema and its application was discontinued after 4 days; a lotion containing 2.5 mg./ml. was tolerated. When a lotion containing 5 mg. BIQ 16 iodide/ml. aqueous alcohol was applied daily to the unbroken skin of 3 human subjects, no reactions were seen.

## DISCUSSION

There was little to choose in antifungal potency between *iso*quinolinium and quinolinium compounds of the same chain-length; but chain-length itself had a considerable effect. The contour of the relation between chain-length and potency for *T. mentagrophytes* (Fig. 1) most nearly resembles that for *Mycobacterium phlei* among bacteria previously examined (Collier *et al.*, 1953). This provides another example of compounds possessing similar antifungal and antimycobacterial properties previously only observed in thioureas by Mayer, Eisman, and Konopka (1953).

The low fungicidal activity of BIQ 16 against spore suspensions in saline was not unexpected in view of the failure of Klarmann and Wright (1954) to detect fungicidal activity in similar tests with conventional quaternary ammonium antibacterials. The striking increase in fungicidal activity with continued exposure to broth may explain the difference in shape of the chain-length-potency contours illustrated in Fig. 1. Readings after 3 days' incubation might measure growth inhibition and those after 14 days' might measure fungicidal activity. These two distinct forms of action might be expected to show different relations to compound chain-length.

TABLE IV

POLYMETHYLENE BISQUINOLINIUM AND BIS*ISO*QUINOLINIUM IODIDES

The reaction solvent was methylethylketone.

$$\left\{R^+-[CH_2]_n-R^+\right\}2\,I^-$$

| Value of *n* | Reaction Time (hr.) | M.p. | Cryst. Form | Crystn. Solvent | Found %* | | | | Formula | Required % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | I | | C | H | N | I |
| Quinolinium | | | | | | | | | | | | | |
| 11 | 104 | 190–192° | Yellow needles or rosettes | EtOH | 52·5 | 5·4 | 3·9 | 37·8 | $C_{29}H_{36}N_2I_2$ | 52·25 | 5 45 | 4 2 | 38·1 |
| 12 | 180 | Rapid: 157–9° Slow: sinters at 157–9° m.p. 186–188° | Yellow needles | EtOH·$Et_2O$ | 53·0 | 5·8 | 4·1 | 37·7 | $C_{30}H_{38}N_2I_2$ | 52 9 | 5·6 | 4·1 | 37·35 |
| 13 | 180 | 170° | Yellow granules | ,, | 53·55 | 5·7 | 4·0 | 35·9 | $C_{31}H_{40}N_2I_2$ | 53·6 | 5·8 | 4·0 | 36·6 |
| 14 | 300 | 157–158° | ,, ,, | ,, | 54·4 | 6·0 | 4·05 | 35·8 | $C_{32}H_{42}N_2I_2$ | 54·2 | 6·0 | 3·95 | 35·9 |
| 16 | 160 | 148–150° | ,, ,, | ,, | 55·1 | 6·55 | 3·8 | 34·3 | $C_{34}H_{46}N_2I_2$ | 55·4 | 6·3 | 3·8 | 34·5 |
| 18 | 120 | 157–158° | Yellow nodules or granules | ,, | 56·7 | 6·6 | 3·6 | 33·3 | $C_{36}H_{50}N_2I_2$ | 56·5 | 6·6 | 3·7 | 33·25 |
| 20 | 240 | 160–161° | Yellow granules | ,, | 57·5 | 6·7 | 3·3 | 31·7 | $C_{38}H_{54}N_2I_2$ | 57·6 | 6·9 | 3·5 | 32·1 |
| *Iso*quinolinium | | | | | | | | | | | | | |
| 14† | 48 | 163–165° | White nodules | ,, | 62·2 | 7·1 | 4·6 | 26·1‡ | $C_{32}H_{42}N_2Br_2$ | 62·5 | 6·9 | 4·6 | 26·1‡ |
| 15 | 24 | 145–146° | Yellow nodules | ,, | 55·0 | 6·0 | 4·0 | 34·5 | $C_{33}H_{44}N_2I_2$ | 54·85 | 6·1 | 3·9 | 35·2 |
| 17 | 24 | 85–86° | Yellow prisms | MeCOMe/$Et_2O$ | 56·3 | 6·65 | 3·6 | 33·8 | §$C_{35}H_{48}N_2I_2$ | 56·0 | 6·45 | 3·7 | 33·9 |
| 19 | 48 | 133–134° | Yellow needles | EtOH/$Et_2O$ | 56·4 | 6·9 | 3·5 | 32·8 | $C_{37}H_{52}N_2I_2$ | 57·1 | 6·7 | 3·6 | 32·65 |

* Dried at 100° *in vacuo*. † Bromide (this was also prepared by double decomposition from the methosulphate). ‡ Bromine.
§ Dried at 56° *in vacuo*.

The compounds BIQ 14 and 16 and BQ 14 and 16 were chosen for further examination, for three reasons: (i) they came close to the peaks of activity against *T. mentagrophytes*; (ii) compounds with an even number of methylene groups were distinctly easier to prepare than the odd, especially those with 15, 17, or 19 methylene groups; and (iii) in the even members of both series acute subcutaneous toxicity in mice increased sharply above the hexadecamethylene compounds. Of these four compounds, BIQ 14 and 16 appeared preferable on the grounds of lower toxicity and greater ease of preparation. The same considerations indicated that BIQ 16* was preferable to BIQ 14. BIQ 16 had the additional advantages of greater activity against *Staphylococcus aureus* and other bacteria that may infect skin (Collier *et al.*, 1953) and against *Candida albicans*. It may be noted in passing that the hexadecamethylene compounds provide an exception to the usual trend towards increased toxicity which accompanies increase in chain-length. When the data obtained in examining the toxicities of BIQ 14 and 16 were considered with those for *in vitro* antifungal potencies, clinical trials seemed justified. The result of a preliminary trial of BIQ 14 in calves was encouraging; trials of BIQ 16 are now in progress.

*Registered name "Teoquil."

## CHEMICAL SECTION

Microanalyses were by Drs. Weiler and Strauss, Oxford. All melting-points are uncorrected.

Most of the polymethylene di-iodides used in this work have already been described, but the following appear to be new: *pentadecamethylene di-iodide*, flat glistening white needles, m.p. 49–50.5°, from alcohol. (Found: C, 39.1; H, 6.5; I, 54.4. $C_{15}H_{30}I_2$ requires C, 38.8; H, 6.5; I, 54.7%); *heptadecamethylene di-iodide*, feathery white needles, m.p. 56–57.5°, from alcohol. (Found: C, 41.8; H, 7.0; I, 51.3. $C_{17}H_{34}I_2$ requires C, 41.5; H, 7.0; I, 51.6%); and *nonadecamethylene di-iodide*, glistening white plates, m.p. 63–64°, from alcohol. (Found: C, 43.8; H, 7.3; I, 48.6. $C_{19}H_{38}I_2$ requires C, 43.85; H, 7.4; I, 48.85%.)

1:17-*Dicyanoheptadecane*, an intermediate in the synthesis of nonadecamethylene di-iodide, is also apparently unrecorded. This crystallized from light petroleum (b.p. 40–60°) in small white leaflets, m.p. 54–55°. (Found: C, 78.4; H, 11.7; N, 9.75. $C_{19}H_{34}N_2$ requires C, 78.6; H, 11.8; N, 9.7%.)

Some of the bis*iso*quinolinium salts investigated have been described in our earlier paper (Collier *et al.*, 1953). The new *polymethylene bisquinolinium* and *bis*isoquinolinium *bromides* and *iodides*, the properties of which are given in Table IV, were prepared by the general method previously described (Collier *et al.*, 1953). As a rule, the *iso*quinolinium salts were more readily purified, and obtained in greater yield, than were the quinolinium compounds.

Other salts were prepared by double decomposition as indicated below.

*Tetradecamethylene bis(*iso*quinolinium methosulphate).*—Tetradecamethylene bis(*iso*quinolinium iodide) [10 g.] and dimethyl sulphate (6 ml.) were heated together at 100° for 15 min. Acetone (200 ml.) was then added, and the mixture refluxed for 30 min. The white product was filtered and recrystallized from a mixture of alcohol and acetone, yielding the required *methosulphate* as white prisms, m.p. 224–225°. (Found: C, 60.2; H, 6.7; N, 4.2; S, 9.4. $C_{34}H_{48}O_8N_2S_2$ requires C, 60.4; H, 7.2; N, 4.1; S, 9.5%.)

Similarly, *tetradecamethylene bis(quinolinium methosulphate)* was obtained as tan-coloured microcrystals of the monohydrate, m.p. 212–213°, from alcohol-ether. (Found: C, 58.9; H, 6.9; N, 4.0; S, 8.95. $C_{34}H_{48}O_8N_2S_2$. $H_2O$ requires C, 58.8; H, 7.3; N, 4.0; S, 9.2%.)

Similarly, *hexadecamethylene bis(*iso*quinolinium methosulphate)* was obtained as clusters of small white needles, m.p. 172°, from alcohol-acetone. (Found: C, 61.0; H, 7.4; N, 3.8; S, 9.1. $C_{36}H_{52}O_8N_2S_2$ requires C, 61.4; H, 7.45; N, 4.0; S, 9.1%.)

*Tetradecamethylene bis(*iso*quinolinium nitrate).*—A solution of the corresponding methosulphate (20 g.) in water (650 ml.) was treated with powdered potassium nitrate (120 g.) with stirring. The white crystalline precipitate was filtered off, dried and recrystallized from alcohol-ether, giving the required *nitrate* as white needles, m.p. 128–129°. (Found: C, 66.3; H, 7.4; N, 9.8. $C_{32}H_{42}O_6N_4$ requires C, 66.4; H, 7.3; N, 9.7%.)

*Tetradecamethylene bis(*iso*quinolinium chloride)* was obtained from the methosulphate in a similar manner, and the dihydrate crystallized from a mixture of alcohol, acetone, and ether as white nodules, m.p. 103–104°. (Found: C, 68.0; H, 8.25; N, 4.8; Cl, 12.65. $C_{32}H_{42}N_2Cl_2$. $2H_2O$ requires C, 68.4; H, 8.3; N, 5.0; Cl, 12.7%.) On drying *in vacuo* at 100°, the anhydrous salt, m.p. 133–134°, was obtained.

*Tridecamethylene bis(*iso*quinolinium perchlorate)* was prepared from a solution of the corresponding iodide (0.5 g.) in warm alcohol (10 ml.) by treatment with 60% aqueous perchloric acid (0.5 g.). The oil, which separated immediately, solidified on scratching to a pale yellow powder, which on recrystallization from methanol-ether yielded the required *perchlorate* as a white micro-crystalline powder, m.p. 128–129°. (Found: C, 58.4; H, 6.2; N, 4.3; Cl, 11.2. $C_{31}H_{40}O_8N_2Cl_2$ requires C, 58.2; H, 6.3; N, 4.4; Cl, 11.1%.)

It was originally proposed to extend this series by preparing polymethylene bisquaternary salts of various alkyl substituted quinolines and *iso*quinolines. Thus, 6-methylquinoline, on refluxing with tetradecamethylene di-iodide in methyl ethyl ketone, yielded *tetradecamethylene bis(6-methylquinolinium iodide)* as yellow-brown prisms, m.p. 133–135°, from methanol-acetone-ether (Found: C, 55.9; H, 6.2; N, 3.6; I, 34.1. $C_{34}H_{46}N_2I_2$ requires C, 55.4; H, 6.3; N, 3.8; I, 34.5%), and 3-methyl*iso*quinoline gave *tetradecamethylene bis(3-methyl*iso*quinolinium iodide) as* pale yellow micro-crystals, m.p. 227–228°, from methanol-ether. (Found: C, 55.4; H, 6.6; N, 3.9; I, 34.55%.)

When, however, tetradecamethylene di-iodide was refluxed with (*a*) quinaldine, (*b*) 7-methylquinoline or (*c*) 1-methyl*iso*quinoline in methyl ethyl ketone, the resultant quaternary salts gave analytical figures corresponding to the loss of two methylene groups from the expected products as indicated below:

(*a*) m.p. 171–172°. Found: C, 54.0; H, 6.1; N, 4.1; I, 35.8.

(*b*) m.p. 160–161°. Found: C, 54.0; H, 6.2; N, 3.9; I, 35.7.

(*c*) m.p. 178–179°. Found: C, 53.9; H, 6.3; N, 3.9; I, 35.8. $C_{32}H_{42}N_2I_2$ requires C, 54.2; H, 6.0; N, 3.95; I, 35.9%.

Obviously, if this had been due to a simple demethylation of the nuclear methyl group, products (*a*) and (*b*) would have had the same melting-point and would have been identical with the previously prepared tetradecamethylene bisquinolinium iodide, and product (*c*) would have been the corresponding bis-*iso*quinolinium iodide. Mixed melting-point determinations showed that this was not so.

No simple explanation of these anomalous results is apparent, but no extensive study of the problem has been made. The alkylquinolines and -*iso*quinolines were purified before use by either recrystallization of a solid derivative or by redistillation. The purified bases all yielded picrates of the correct melting-point.

## Summary

1. Polymethylene bis*iso*quinolinium compounds with 15, 17, and 19 methylene groups and a series of bisquinolinium salts with 11–14, 16, 18, and 20 methylene groups have been prepared.

2. Polymethylene bis*iso*quinolinium salts with 10–20 methylene groups and the corresponding quinolinium salts were examined for activity against *T. mentagrophytes*. In both series, activity increased with increase in chain-length up to the tetradecamethylene member: a marked decline in activity was seen at the eicosane compounds.

3. The tetradeca- and hexadecamethylene members of both series were found to inhibit 11 strains of 6 species of pathogenic fungi in Sabouraud's broth at concentrations between 0.3 and 10 μg./ml.

4. The tetradeca- and hexadecamethylene bis*iso*quinolinium salts were slightly antagonized by hair and serum and the latter compound was potently antagonized by bovine bile.

5. Exposure for 24 hr. at 20° C. to hexadecamethylene bis(*iso*quinolinium methosulphate) did not destroy spores of *T. mentagrophytes* suspended in saline. On the other hand, viable spores could not be detected after incubation for 7 days at 27° C. with a concentration of 1.25 μg./ml. of this compound in Sabouraud's broth.

6. The acute subcutaneous toxicities of the four tetradeca- and hexadecamethylene compounds and the acute oral toxicities of the two *iso*quinolinium compounds were determined in mice. Observations were also made on the acute intradermal toxicities and on the subacute toxicities to eyes and skin of rabbits.

7. The results appeared to warrant clinical trial of the tetradeca- and hexadecamethylene bis*iso*-quinolinium compounds in fungal infections of the skin.

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