Antidermatophytic Properties of Griseofulvin Derivatives with Potential Systemic Insect-Repellent Activity

ROBERT G. CROUNSE

Abstract □ The antidermatophytic properties of a number of grisan and coumaranone derivatives, designed as potential systemic insect repellents, have been evaluated *in vitro*. Comparisons of their antimycotic efficacy with the corresponding *in vitro* activity of established antidermatophytic agents (*e.g.*, griseofulvin and aliphatic carboxylic acids) have been made. Several derivatives were active antifungal agents, though of lesser potency than griseofulvin itself. Differing species specificity suggests differences in antimycotic properties and might allow individual molecular design and selection for enhanced inhibition of a given dermatophytic species.

Keyphrases □ Antidermatophytic agents—griseofulvin derivatives □ Griseofulvin derivatives—antimycotic, antifungal activity □ Agar plate, liquid shake cultures—assay methods

The synthesis and physicochemical evaluation of a number of grisan and coumaranone derivatives designed as potential systemic insect repellents have been reported (1–3). In this communication, the *in vitro* antidermatophytic properties of these compounds were evaluated with the expectation that the data could provide a basis for their quantitative determination in dermal tissue. The antidermatophytic efficacy of griseofulvin, some related grisan and coumaranone derivatives, and that of octanoic, decanoic, and undecanoic acids was also determined for comparison with the

Table I—Comparison of Percent Inhibition of Radial Plate *versus* Pellet Growth, *T. mentagrophytes*, 6 Days Growth

Compound	Concn.	Pellet	Plate
I	5	88	95
I*a*	10	28	38
	20	52	25
I*b*	10	28	45
	20	40	95
I*c*	10	52	33
	20	64	30
I*d*	10	4	25
	20	40	25
II	10	0	0
	20	4	38
II*a*	10	28	25
	20	64	65
II*b*	10	28	25
	20	40	40

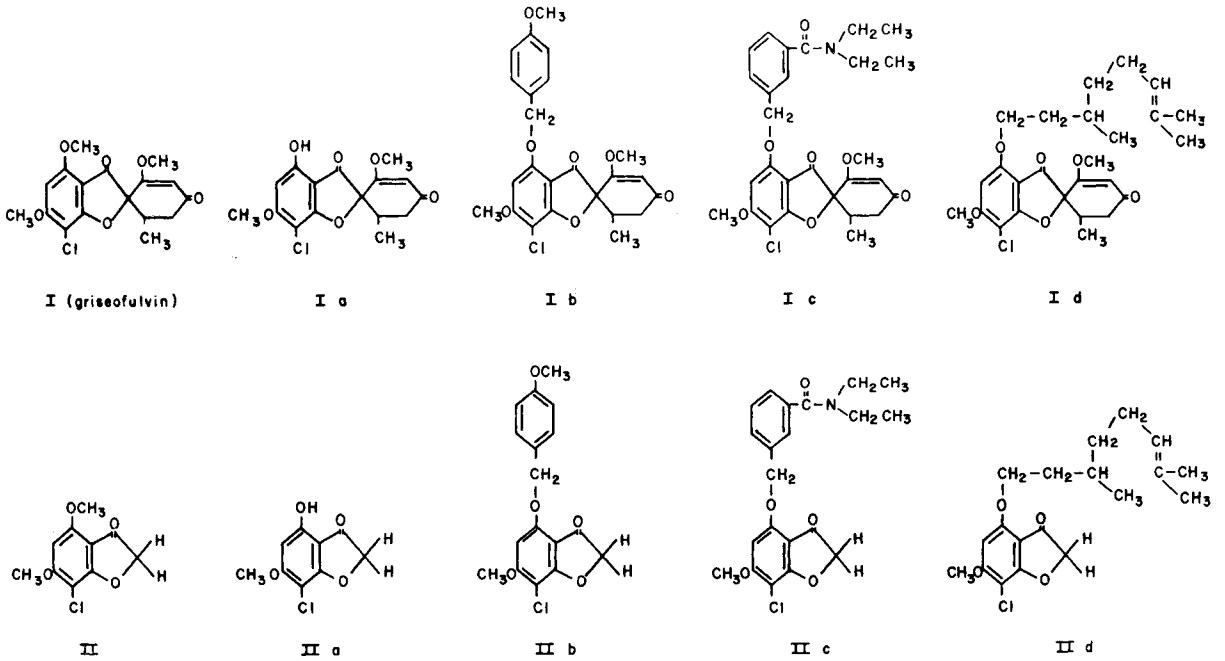

Figure 1—*Evaluant compounds (I): I, griseofulvin; Ia, 4-demethylgriseofulvin; Ib, 7-chloro-6,2'-dimethoxy-4-(p-methoxybenzyloxy)-6'-methylgris-2'-ene-3,4'-dione; Ic, 7-chloro-4-[3-(N,N-diethylcarbamoyl)benzyloxy]-6,2'-dimethoxy-6'-methylgris-2'-ene-3,4'-dione; Id, 7-chloro-6,2'-dimethoxy-4-(3,7-dimethyl-6-octenyl-1-oxy)-6'-methylgris-2'-ene-3,4'-dione; II, 7-chloro-4,6-dimethoxycoumaran-3-one; IIa, 7-chloro-4-hydroxy-6-methoxycoumaran-3-one; IIb, 7-chloro-6-methoxy-4-(p-methoxybenzyloxy)coumaran-3-one; IIc, 7-chloro-4-[3-(N,N-diethylcarbamoyl)benzyloxy]-6-methoxy-coumaran-3-one; IId, 7-chloro-6-methoxy-4-(3,7-dimethyl-6-octenyl-1-oxy)coumaran-3-one.*

Table II—Percent Inhibition of Radial Growth in Plate Culture at 6 Days (error < 10%)

Compound →	I	Ia		Ib		Ic		Id		II		IIa		IIb		IIc		IId	
Concn., mcg./ml. →	5	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20	10	20
T. mentagrophytes	90	25	40	45	95	35	30	25	40	0	40	25	65	25	40	0	0	0	0
T. rubrum	96	10	15	0	10	36	54	25	25	0	0	0	58	0	75	37	32	40	40
M. gypsum	100	68	—	0	0	43	46	17	28	0	0	26	30	22	17	—	—	—	—
K. ajelloi	0	4	25	25	85	81	79	4	25	2	20	—	64	25	45	—	—	—	—

Table III—Percent Inhibition[a] of Growth in Plate Culture at 14 Days

	Compd.									
	I	IIIa	IIIb	IIIc	IVa	IVb	IVc	Va	Vb	Vc
E. floccosum	64	11	33	31	21	40	32	0	37	25
M. canis	92	1	50	45	4	68	66	16	58	52
T. rubrum	89	7	52	49	8	42	67	0	59	59
T. mentagrophytes	65	0	63	67	2	57	63	4	68	34

[a] Average of two separate experiments (error < 10%); griseofulvin concentration is 5 mcg./ml., others 20 mcg./ml.

activity of the novel compounds. The structures of the evaluants are depicted in Figs. 1 and 2.

MATERIALS AND METHODS

The preparation and properties of the grisan and coumaranone evaluant compounds have been described (1–3); the samples employed[1] were of analytically pure grade or the equivalent. Octanoic acid (Eastman), and decanoic and undecanoic acids (K&K) were utilized without further purification.

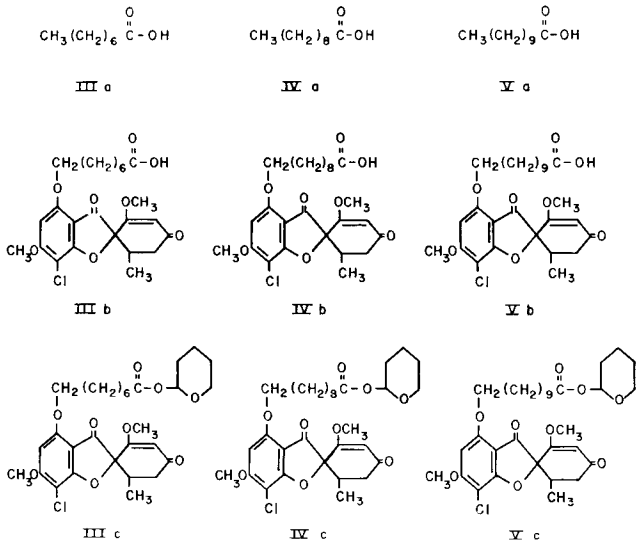

Figure 2—*Evaluant compounds (2): IIIa, octanoic acid; IIIb, 4 - (7 - carboxyheptyl - 1 - oxy) - 7 - chloro - 6,2' - dimethoxy - 6'-methylgris-2'-ene-3,4'-dione; IIIc, tetrahydropyran-2-yl ester of 4 - (7 - carboxyheptyl - 1 - oxy) - 7 - chloro - 6,2' - dimethoxy - 6'-methylgris-2'-ene-3,4'-dione; IVa, decanoic acid; IVb, 4-(9-carboxynonyl - 1 - oxy) - 7 - chloro - 6,2' - dimethoxy - 6' - methyl - gris-2'-ene-3,4'-dione; IVc, tetrahydropyran-2-yl ester of 4-(9-carboxynonyl - 1 - oxy) - 7 - chloro - 6,2' - dimethoxy - 6' - methyl - gris-2'-ene-3,4'-dione; Va, undecanoic acid; Vb, 4-(10-carboxy-decyl - 1 - oxy) - 7 - chloro - 6,2' - dimethoxy - 6' - methylgris - 2' - ene-3,4'-dione; Vc, tetrahydropyran-2-yl ester of 4-(10-carboxy-decyl - 1 - oxy) - 7 - chloro - 6,2' - dimethoxy - 6' - methylgris - 2' - ene-3,4'-dione.*

[1] All of the evaluants were provided by Drs. R. P. Quintana and A. Lasslo, Dept. of Medicinal Chemistry, College of Pharmacy, University of Tennessee Medical Units, Memphis, Tenn.

Clinical isolates of *Trichophyton rubrum* and *mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis* and *gypseum* were maintained in serial culture by standard techniques. A single strain of *Keratinomyces ajelloi* was also utilized in some studies. Estimation of radial growth per unit time was assayed both in liquid shake cultures and in standard Sabouraud's agar circular culture plates.

Radial growth was measured in millimeters with a dissecting microscope in the case of the pellet growth in shake culture, and by direct observation in the case of plate growth. All experiments were done in duplicate or triplicate and repeated at least once. Appropriate controls were included in every experiment. Test compounds were included in the media in concentrations of 5, 10, and 20 mcg./ml. All of the preparations appeared, visually, to be homogeneous.

RESULTS AND DISCUSSION

The results of a series of evaluations comparing pellet *versus* plate growth inhibition are shown in Table I. Though generally similar results were obtained, the pellet technique was found to be more erratic, and was discarded eventually in favor of the plate method. An example of the relative inhibitory properties of several compounds for a given fungal species is illustrated in Fig. 3. The inhibition in all instances appeared to be effected through slowing the growth (in terms of radial spread) as can be seen from the graph. A summary of all of the experimental results is included in Tables II and III. Although none of the compounds tested exhibited antidermatophytic activity equal to that of griseofulvin at the lower concentration, several of them exhibited significant antidermatophytic activity at slightly higher concentration levels. Moreover, differences in absorption, distribution, cutaneous localization, and binding might significantly favor one of the derivatives such that its *in vivo* biologic activity might be comparable to, or perhaps better than, that of griseofulvin itself. Elucidation of these parameters must await further investigation. Additional factors which may affect the results include the solubilities of the respective compounds and their penetration into the fungal cell wall.

In terms of structure–activity relationships (*cf.* 4), the poor inhibitory efficacy of Compound II is interesting; the removal of the

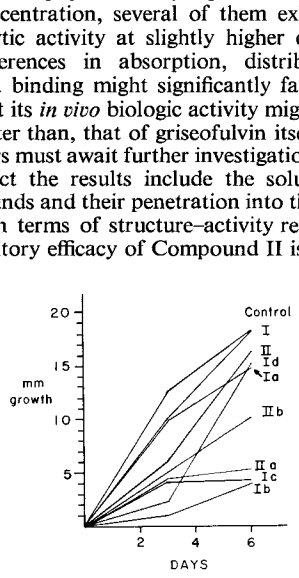

Figure 3—*Inhibition of growth rate of* K. ajelloi *(plate culture) by a series of synthetic analogs* (cf. *Fig. 1). This organism was resistant to griseofulvin (I) in ordinary concentrations (see text).*

third ring component in griseofulvin is associated with a dramatic reduction of *in vitro* antidermatophytic activity.

Direct comparison of the fatty acid–griseofulvin derivatives (III*b*, IV*b*, V*b*) with the corresponding fatty acids (III*a*, IV*a*, V*a*) (Table III) indicated significantly enhanced antidermatophytic properties of the former entities in excess of the known and measured inhibitory properties of the individual fatty acids. However, none of the new compounds was as effective, on a microgram basis, as griseofulvin.

Several species differences could be demonstrated (Tables II and III). Compound I*b* (Table II), for example, was severely inhibitory for *T. mentagrophytes*, but was not at all effective against *M. gypseum* or *T. rubrum*. The single strain of *K. ajelloi* utilized in these experiments was quite resistant to amounts of griseofulvin which normally inhibit pathogenic dermatophytes (Table II). It was, however, inhibited by several of the griseofulvin derivatives at higher concentration. The isolate used was obtained from soil; it has been reported that soil isolates are relatively resistant to griseofulvin in contrast to pathogenic strains from clinical infections which tend to be griseofulvin sensitive (5). It may be added that definite species differences in the sensitivity to various compounds is of major interest in terms of the ultimate tailoring of a molecule for a specific effect.

The general concept of a skin-directed molecule also incorporating moieties for different therapeutic effects can, of course, be extended to several other situations such as those requiring antibacterial, antiyeast, and anti-inflammatory activities. Success from such an approach may well be based upon the synthesis of a graded series of derivatives such as those reported in this communication.

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Determination of 2-Chloroethanol in Surgical Materials by Extraction and Gas Chromatography

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Abstract □ A method for the determination of 2-chloroethanol in ethylene oxide sterilized surgical materials is described. The 2-chloroethanol is extracted under vacuum, condensed in a cold trap, and quantitative determinations made by GLC. The method is compared with acetone and water extractions.

Keyphrases □ Ethylene oxide sterilized plastic, rubber—2-chloroethanol determination □ 2-Chloroethanol extraction, determination—surgical materials □ GLC—analysis

Methods for the determination of 2-chloroethanol (ethylene chlorohydrin) in foods, plastic, and rubber have recently appeared in the literature (1–5). We have found these methods to be unreliable for this determination in polyvinyl chloride plastic (PVC), and in synthetic and latex rubber.

Cunliffe and Wesley (2) used saline and blood extraction to demonstrate the formation of 2-chloroethanol in solution by surgical plastics sterilized with ethylene oxide. None was found when distilled water was used. They did, however, find 2-chloroethanol in irradiated PVC which was subsequently sterilized with ethylene oxide. Irradiation formed HCl within the PVC which reacted with the ethylene oxide. This demonstrated that either absorbed ethylene oxide reacted with Cl^- within the plastic-forming 2-chloroethanol, or that the reaction took place in the elution liquid. The formation of 2-chloroethanol, due to Cl^- in PVC which had not been irradiated, was not demonstrated. It cannot be clearly shown by their method that 2-chloroethanol was formed during the sterilization process, but only that irradiation prior to ethylene oxide exposure resulted in the presence of detectable quantities of 2-chloroethanol in an elution solvent (*i.e.*, H_2O).

A method has been developed which appears to be reliable for determining 2-chloroethanol in a variety of materials. Extraction of 2-chloroethanol is achieved by heat and high vacuum distillation with collection in a U tube cooled in liquid nitrogen. The collected 2-chloroethanol was quantitatively determined by GLC. This method was compared with other reported techniques for determination of 2-chloroethanol.

EXPERIMENTAL

Apparatus—A 250-mlg. round-bottom flask with 24/40 ground-glass joint; glass tube with ground fitting for attachment to flask; fitting (Swagelock) to adapt glass tube to steel; stainless steel U tube, 0.305 m. length × 0.635 cm. o.d. (1 ft. length × 0.25 in. o.d.); vacuum source capable of less than forty μ. Gas chromatography was performed on an instrument (Perkin Elmer model 800) equipped with a dual-flame ionization detector. Also used was a column [0.318 cm. × 1.829 m. (1/8 in. × 6 ft.) ss 10% polyethylene glycol (Carbowax 1540) on Teflon 35M].