ANTIMICROBIAL ACTIVITY OF SESQUITERPENE LACTONES

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Twenty-two sesquiterpene lactones isolated from Asteraceae species were evaluated for antimicrobial activity against bacteria and fungi. Fourteen compounds showed activity, chiefly against Gram positive bacteria, being two of them, dehydroleucodin and 3,4-epoxyleucodin (guaianolides) active against Gram positive and Gram negative bacteria, yeasts and dermatophytes.

INTRODUCTION

Sesquiterpene lactones are biologically active compounds. They are known to poison livestock[1], to act as insect feeding deterrents[1,2] and to cause contact dermatitis in humans[3]. They also can inhibit the penetration of cercariae of *Schistosoma mansoni*[4,5] or microbial growth[6-9], and exhibit cytotoxic[10], antitumoral[11], or antiinflammatory[12] activities, among others[1,13,14].

Sesquiterpene lactones are isoprenoid metabolites, with more than 2500 reported compounds and occur chiefly in members of Asteraceae where they are characteristic constituents of the family (except the tribe Tagetae), although they also occur sporadically in other angiosperm families[15].

In the present study 22 sesquiterpene lactones (*1-22*), isolated from Asteraceae, were tested against bacteria and fungi.

MATERIAL AND METHODS

The microorganisms studied were: *Bacillus cereus* ATCC 14579 (B.c.); *Staphylococcus aureus* ATCC 6538 (S.a.); *Staphylococcus epidermis* ATCC 12228 (S.e.); *Escherichia coli* ATCC 25922 (E.c.); *Klebsiella pneumoniae* ATCC 10031 (K.p.); *Candida albicans ICB 12 (C.a.); Candida glabrata* ICB 51 (G.g.); *Candida tropicalis* ICB 19 (C.t.); *Cryptococcus neoformans* ICB 59 (C.n.); *Cladosporium sphaerospermum* SBC 491 (C.s.); *Aspergillus flavus ICB J-210; Microsporum gypseum* ICB A-02; *Trichophyton rubrum* ICB B-2-11.

The cup-plate agar diffusion method[16] was used to test the compounds against bacteria and yeasts, in a concentration of 0.5 mg/ml. The plates were incubated at 37°C and the activities measured as the diameters (in mm) of the zone of inhibition surrounding the agar well, after 24 and 48 hs.

A two-fold serial dilution method in liquid medium[16] was employed for antifungal testing with dermatophytes and *Aspergillus flavus*, the concentration of samples ranging from 500 to 15.6 ug/ml.

Antifungal activity was also accomplished using a bioautography assay[17]. Samples containing 50 ug of the test compound were spotted on silica gel plates (Alufolien, Merck) and spores of *Cladosporium sphaerospermum* suspended in a medium containing glicose and mineral salts were sprayed onto the plates. Inhibitory zones on the plates appeared as white zones on a dark background, after 72 hr of incubation in a moist atmosphere at 25°C.

RESULTS AND DISCUSSION

The results of assays by agar diffusion methods and bioautography are summarized in Table I.

Fourteen of the lactones tested were active against Gram positive bacteria. Only compounds *1* and *2* showed good activity against the Gram negative ones and yeasts.

It is important to note that compounds *15* and *16* were isolated and assayed as a mixture.

Only few compounds inhibited the growth of dermatophytes and *Aspergillus flavus*.

The results for antifungical testing (minimum inhibitory concentration in ug/ml) were as follows:

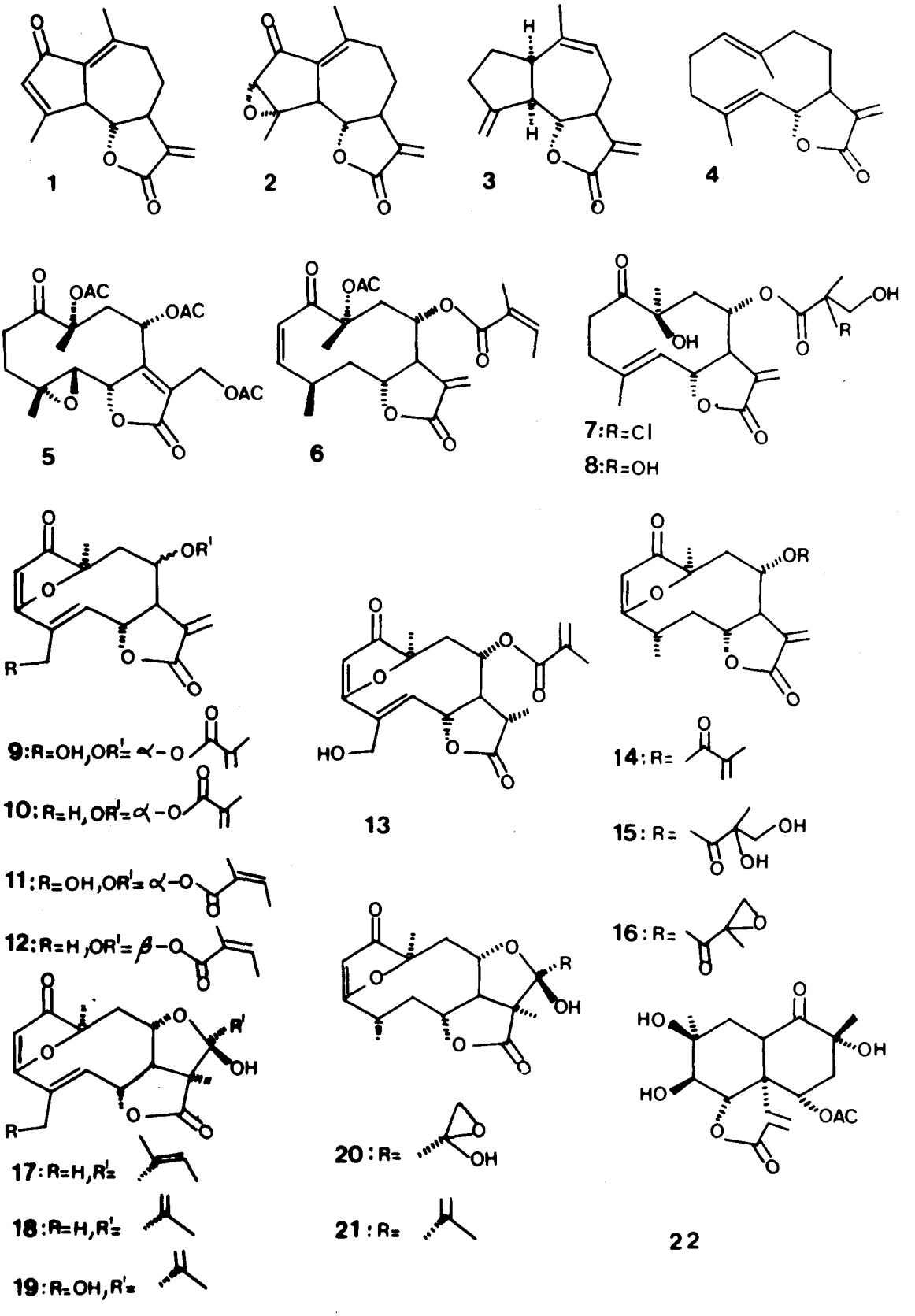
A. flavus: compound *1* (250); *2* (250).

M. gypseum: compound *1* (15); *2* (62,5); *3* (125); *4* (500); *6* (500); *12* (125); *18* (125).

T. rubrum: compound *1* (31,2); *2* (62,5); *3* (125); *4* (500); *6* (500); *12* (125).

There have been several reports of antibacterial activity of individual sesquiterpene lactones. The majority of results showed that they can inhibit the growth of Gram positive and negative bacteria but Gram positive ones were more affected[13]. Screening tests of these compounds against *C. albicans, T. mentagrophytes* and *M. cookei* showed that few sesquiterpene lactones can inhibit the yeast[13], but many guaianolides (like compounds *1*, *2* and *3*,), among other active compounds, particularly eudesmanolides, can inhibited or reduce the growth of the dermatophytes[18]. The findings are in agreement with our results.

Several studies tried to establish a relationship between antimicrobial activity and structure of sesquiterpene lactones. Calzada et al.[7] investigated the antimicrobial activity of heliangolides and concluded that there was a clear dependence between activity and the presence of an α-methylene-γ-lactone group.



Lee et al.[6], after testing 36 compounds, have suggested that the activity is associated with the presence of a β-unsubstituted cyclopentene ring.

In our case it is not easy to find a structure-activity relationship, as many structural types were studied and the number of compounds is limited. Anyway the results showed that the effectiveness of the lactones was not correlated with the presence of either an α-methylene-γ-lactone residue or a β-unsubstituted cyclopentenone ring. Other factors must play a role in determining the antimicrobial activity. This agrees with

Table I. Antimicrobial activity of sesquiterpene lactones (500 μg/ml) as determined by the agar diffusion method and bioautography.

COMPOUND	MICROORGANISM									
	B.c.	S.a.	S.e.	E.c.	K.p.	C.a.	C.t.	C.g.	C.n.	C.s.
[*1*]	12	15	13	10	11	10	8	–	14	+
[*2*]	20	20	18	9	11	10	–	–	10	+
[*3*]	–	–	–	–	–	–	–	–	–	–
[*4*]	–	–	–	–	–	–	–	–	–	+
[*5*]	10	12	9	–	–	–	–	–	–	+
[*6*]	18	13	11	–	–	–	–	–	–	–
[*7*]	16	18	15	–	–	–	–	–	–	–
[*8*]	14	13	10	–	–	–	–	–	–	+
[*9*]	17	23	20	–	–	–	–	–	–	+
[*10*]	–	–	–	–	–	–	–	–	–	–
[*11*]	10	10	9	–	–	–	–	–	–	–
[*12*]	12	18	13	–	–	–	–	–	–	+
[*13*]	16	11	15	–	–	–	–	–	–	+
[*14*]	12	–	–	–	–	–	–	–	–	–
[*15 + 16*]	–	–	–	–	–	–	–	–	–	–
[*17*]	12	14	9	–	–	–	–	–	–	–
[*18*]	–	–	–	–	–	–	–	–	–	–
[*19*]	13	12	16	–	–	–	–	–	–	+
[*20*]	–	–	–	–	–	–	–	–	–	–
[*21*]	11	12	9	–	–	–	–	–	–	+
[*22*]	–	–	–	–	–	–	–	–	–	–

Results given as the mean inhibition zone diameter ($n \geq 3$) in mm.
– no inhibition.
+ presence of inhibition zone on biochromatograms.

studies from Picman and Towers[9] who suggested that the various additional groups and their position and configuration on the skeleton may enhance or reduce the activity.

It is interesting to note that small structural changes, as the introduction of an OH group on C-15 (compounds *9, 10* and *18, 19*), or the substitution of a metacrylate for an angelate residue (compounds *17, 18*) can change drastically the antibacterial activity.

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