Notes

GALBONOLIDES A AND B — TWO NON-GLYCOSIDIC ANTIFUNGAL MACROLIDES

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In search for new secondary metabolites of microbial origin, the galbonolides were detected in the fermentation broth of a *Streptomyces* strain. In this note the fermentation, the isolation, the physico-chemical properties and the biological activities of galbonolides A and B are reported.

Taxonomic Studies

The microorganism (Tü 2253) used in this study was isolated from a soil sample collected in Tunisia. According to HÜTTER[1], NONOMURA[2] and BERGEY's Manual[3] the antibiotic producing strain was classified as *Streptomyces galbus*.

Fermentation

S. galbus Tü 2253 was maintained on agar slants and used to inoculate 500-ml Erlenmeyer flasks with one intrusion, containing 100 ml production medium, that consisted of yeast extract 0.4%, malt extract 1%, glucose 0.8% and L-isoleucine 1 mM, pH 7.3. *S. galbus* Tü 2253 was cultured for 48 hours at 27°C on a rotary shaker. Then one liter of culture was used to inoculate a 10-liter fermentor, containing 9 liters of production medium. Fermentation was carried out at 27°C, 300 rpm agitation and 60 liters/minute aeration. To prevent foaming, polyol antifoam was added. After 24 hours of incubation the content of the 10-liter fermentor was transfered into a 100-liter fermentor containing 90 liters of production medium and run at 27°C, 230 rpm agitation and 60 liters/minute aeration. During the fermentation process samples were taken for measuring pH, dry mycelial weight as described by KAPPNER *et al.*[4], and antibiotic concentration by the agar disc-diffusion test[5].

Biological Assay

The agar disc-diffusion test was used for measuring antibiotic activity of the cultures and biological activity during isolation and purification of the galbonolides. As test organism *Botrytis cinerea* was used. The antifungal spectra was also determined by agar disc-diffusion tests.

Isolation and Purification

The content of a 100-liter fermentor was harvested 42~45 hours after inoculation, when the pH of the culture reached 6.0. The culture broth was mixed with 2% Celite and filtered. The mycelium was extracted with methanol and the culture filtrate with ethyl acetate. The extracts were concentrated to aqueous residues, and the residues extracted with dichloromethane and concentrated to give crude extracts. Both extracts were chromatographed separately on 200 g Sephadex LH-20 (Pharmacia, Uppsala) with methanol followed by partition chromatography using a system consisting of 200 g Sephadex LH-20, methanol and petroleum ether (50~70°C) to yield crude galbonolide A (from mycelium and culture filtrate extracts) and crude galbonolide B (from mycelium only). Further purification of both galbonolides was afforded by successive column chromatography on 400 ml Fractogel TSK HW40(S) (Merck, Darmstadt), eluted with methanol, and on 17 g Fractogel PVA-500 (Merck), eluted with ethyl acetate. All separations were done at +2°C. Both galbonolides were crystallized from petroleum ether at −20°C.

Isolation

For the isolation of large amounts of galbonolides A and B fermentation was performed on a 100-liter scale. The course of fermentation is shown in Fig. 1.

The production of galbonolides A and B is

Fig. 1. Time course of fermentation by *S. galbus* Tü 2253.

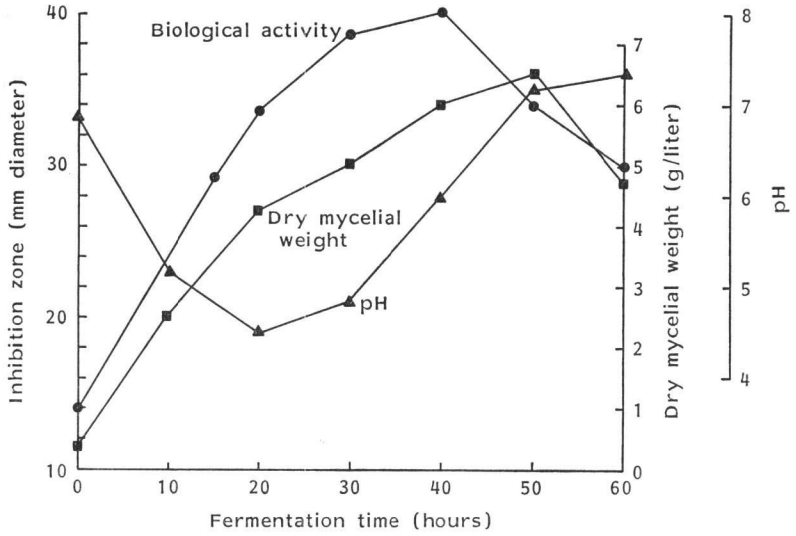
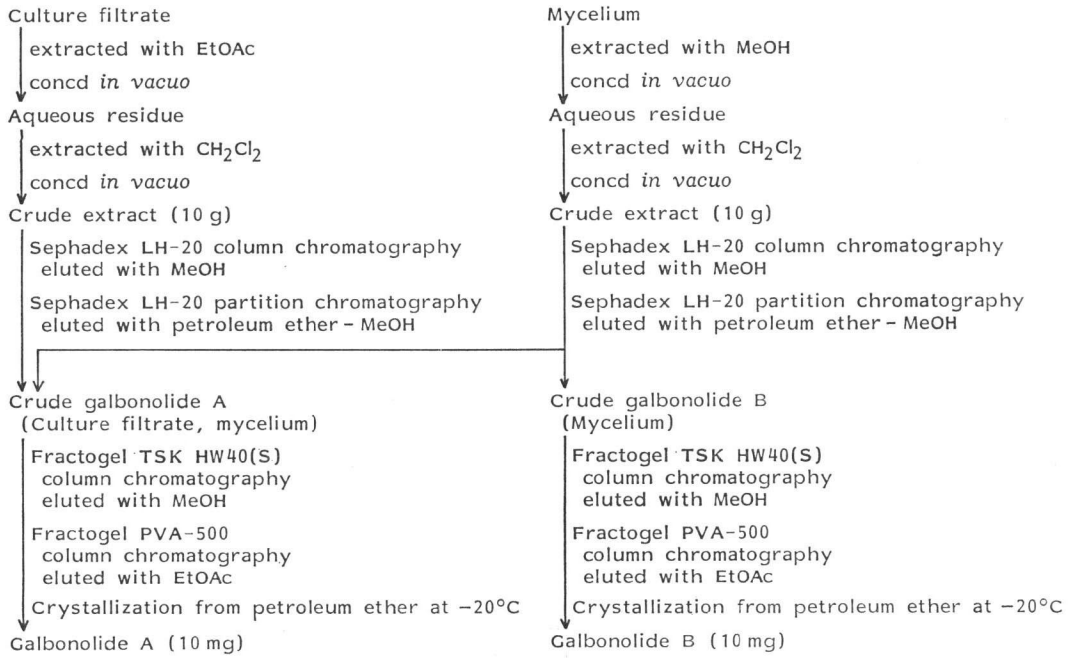


Fig. 2. Flow diagram of the isolation and purification of galbonolides A and B.

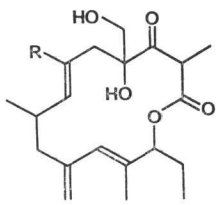


strictly correlated with the increase of dry mycelial weight. Maximal amount of production was attained 40~45 hours after inoculation. Then antibiotic activity decreased as pH increased. For the isolation the culture was harvested at maximal antibiotic activity, when the pH of the culture was 6.0. The flow diagram of the isolation and purification of galbonolides A and B is shown in Fig. 2.

Physical and Chemical Properties

Galbonolides A and B crystallize as white needles (mp 68°C and 109~112°C, respectively); they are fairly soluble in methanol, acetone, ethyl acetate, dichloromethane, chloroform and

Fig. 3. Structures of galbonolides A and B.



Galbonolide A R = OCH_3
Galbonolide B R = CH_3

Fig. 4. UV spectrum of galbonolide A.

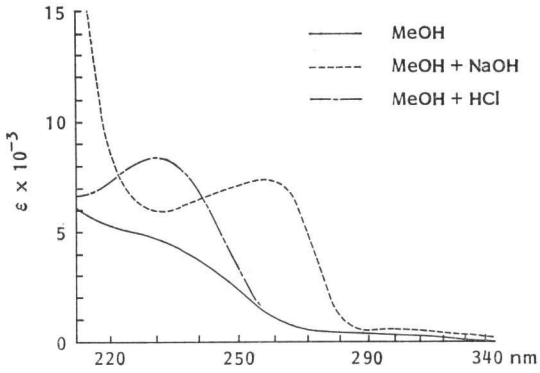


Fig. 5. IR spectrum of galbonolide A in KBr.

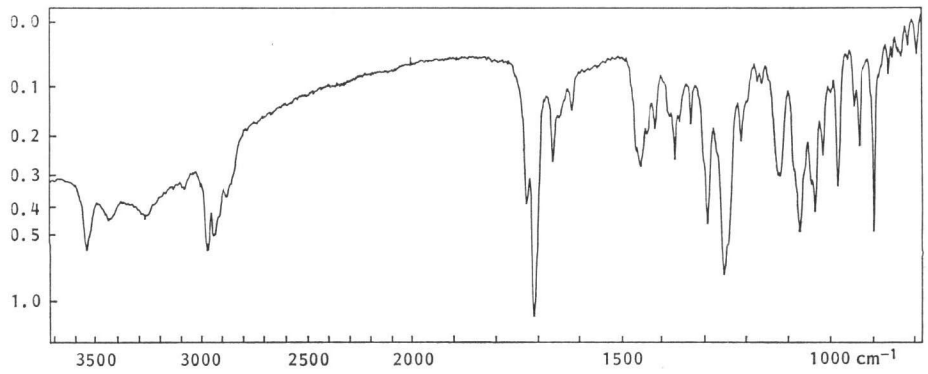


Fig. 6. ^1H NMR spectrum of galbonolide A in acetone-d_6 (400 MHz).

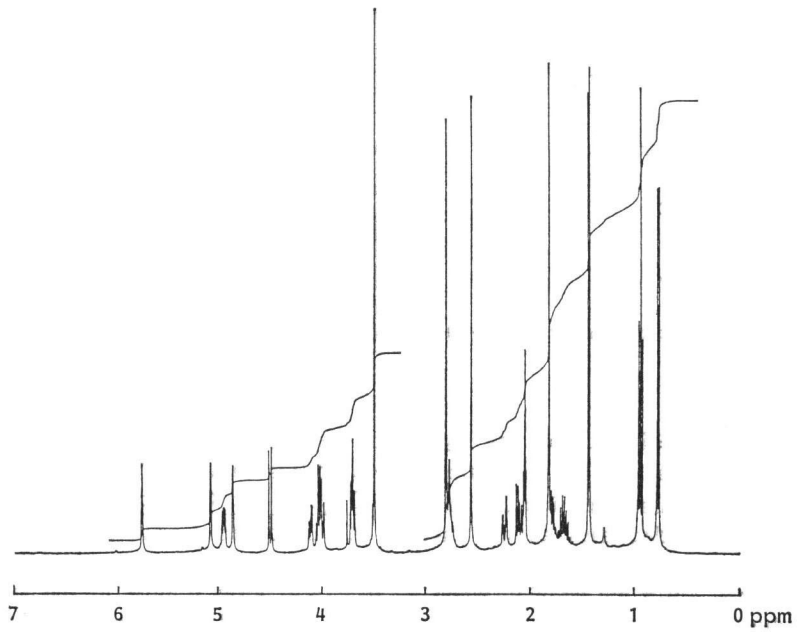


Table 1. The biological activity of the galbonolides against various fungi.

Organisms	Antifungal activity (10 μl/paper disc)	
	Galbonolide A (μg/ml)	Galbonolide B (μg/ml)
Botrytis cinerea	0.1	10
Candida albicans	3	300
C. vulgaris	10	1,000
Cladosporium herbarum	0.1	10
Coprinus cinereus	30	1,000
Endomyces magnusei	1	30
Fusarium solani	10	300
Hansenula anomala	0.3	30
Paecilomyces varioti	300	300
Penicillium notatum	300	—
P. puberulum	1,000	—
Pichia farinosa	0.1	3
Rhizoctonia solani	0.3	10
Rhodotorula rubra	0.1	10
Saccharomyces cerevisiae	3	1,000
Saprolegnia asterophora	1,000	1,000
Sordaria macrospora	300	300
Talaromyces flavus	100	1,000
Ustilago maydis	10	10
Wingea robertsii	10	—

—: No activity at a concentration of 1 mg/ml.

petroleum ether, but insoluble in water. The stability in aqueous solution is highest at pH 6.0; treatment by acid or alkali causes rapid decomposition. Galbonolides A and B are non-glycosidic macrolides with a 14-membered lactone ring. The structures (Fig. 3) were elucidated mainly by spectroscopic studies[6]. The UV, IR and ^{1}H NMR spectra are shown in Figs. 4, 5 and 6.

Biological Activity

The antimicrobial activities of galbonolides A and B against various bacteria and fungi were tested in the agar disc-diffusion assay. As shown in Table 1, the galbonolides exhibited biological activity against a broad spectrum of fungi, whereas the Gram-positive and Gram-negative bacteria tested were not sensitive.

The following organisms were insensitive to both galbonolides at a concentration of 1 mg/ml:

Fungi: *Alternaria kikuchiana*, *Arthrinium phaeospermum*, *Aspergillus fumigatus*, *Byssochlamis nivea*, *Claviceps viridis*, *Microsporum canis*, *Mucor hiemalis*, *Mucor miehei*, *Mycotypha africana*, *Nematospora coryli*, *Phytophthora palmivora*, *Pythium debaryanum*, *Piricularia oryzae*, *Schizosaccharomyces pombe*, *Trichophyton mentagrophytes*.

Bacteria: *Agrobacterium tumefaciens*, *Bacillus brevis*, *Bacillus cereus*, *Clostridium pasteurianum*, *Corynebacterium rathayi*, *Erwinia carotovora*, *Halobacterium cutirubrum*, *Klebsiella aerogenes*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Serratia marcescens*, *Staphylococcus aureus*, *Streptomyces viridochromogenes*.

Furthermore, the galbonolides caused morphological alterations in the hyphal growth of *B. cinerea* (Figs. 7 and 8).

Fig. 7. *Botrytis cinerea* (control).

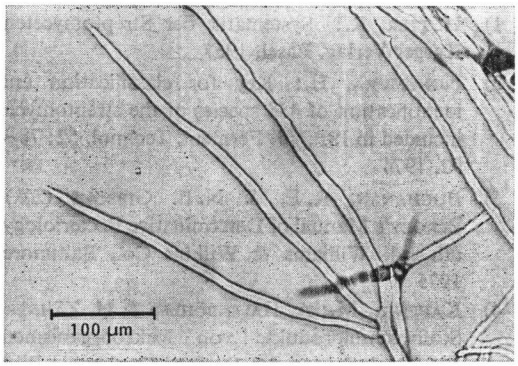
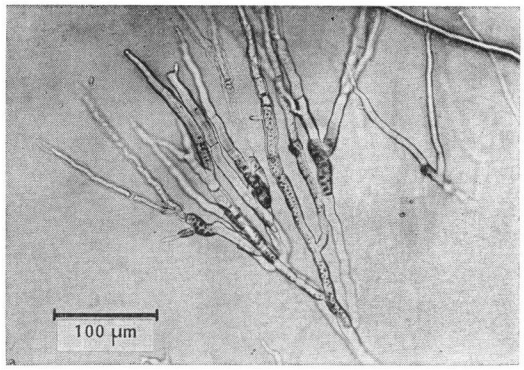


Fig. 8. Abnormal branching of growing hyphae of *Botrytis cinerea* in presence of galbonolide A.



The recently published macrolide antibiotics rustmicin[7] and neorustmicin A[8] isolated from two *Micromonospora* species, are reported to have the same structures as galbonolide A and galbonolide B, respectively.

Acknowledgment

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References

1) HÜTTER, R.: Systematik der Streptomyceten. Karger Verlag, Basel, 1967
2) NONOMURA, H.: Key for classification and identification of 458 species of the streptomyces included in ISP. J. Ferment. Technol. 52: 78~92, 1974
3) BUCHANAN, R. E. & N. E. GIBBONS (*Ed.*): BERGEY's Manual of Determinative Bacteriology. 8th Ed. Williams & Wilkins Co., Baltimore, 1975
4) KAPPNER, M.; A. HASENBÖHLER & H. ZÄHNER: Stoffwechselprodukte von Mikroorganismen. 166. Optimierung der Desferri-Ferricrocin-Bildung bei *Aspergillus viridi-nutans* Ducker & Thrower. Arch. Microbiol. 115: 323~331, 1977
5) HÜTTER, R.; W. KELLER-SCHIERLEIN, J. NÜESCH & H. ZÄHNER: Stoffwechselprodukte von Mikroorganismen. 48. Mitteilung. Scopamycine. Arch. Microbiol. 51: 1~8, 1965
6) ACHENBACH, H.; A. MÜHLENFELD, U. FAUTH & H. ZÄHNER: Galbonolides A and B — two new non-glycosidic antifungal macrolides from *Streptomyces galbus*. Tetrahedron Lett. 26: 6167~6170, 1985
7) TAKATSU, T.; H. NAKAYAMA, A. SHIMAZU, K. FURIHATA, K. IKEDA, K. FURIHATA, H. SETO & N. ŌTAKE: Rustmicin, a new macrolide antibiotic active against wheat stem rust fungus. J. Antibiotics 38: 1806~1809, 1985
8) ABE, Y.; H. NAKAYAMA, A. SHIMAZU, K. FURIHATA, K. IKEDA, K. FURIHATA, H. SETO & N. ŌTAKE: Neorustmicin A, a new macrolide antibiotic active against wheat stem rust fungus. J. Antibiotics 38: 1810~1812, 1985