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AN ANTITUMOR PRINCIPLE FROM *SUILLUS GRANULATUS*

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ABSTRACT.—An antitumor principle from *Suillus granulatus* has been identified as suillin [**1**]. In vitro and in vivo activities against P-388 leukemia have been determined.

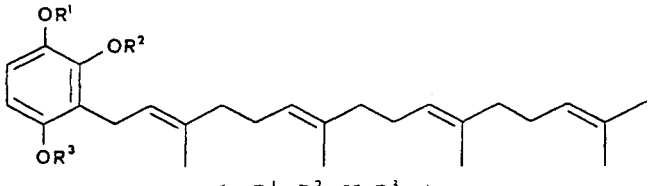
In the search for bioactive compounds from natural sources, we examined the fruiting bodies of several species of Basidiomycetes from the Sicilian flora for cytotoxic and antibacterial activity. The product from a CH_2Cl_2 extract of *Suillus granulatus* (L. ex Fr.) O. Kuntze (Boletaceae) displayed activity against KB cells (ID_{50} = 10.5 μg/ml) and weak activity against *Staphylococcus aureus*. Chromatographic fractionation of the extract guided by biological assays showed that cytotoxic and antibacterial activity were not located in the same fractions; significant cytotoxicity (ID_{50} = 4.2 μg/ml) was detected in one of the fractions, and this prompted us to purify the active principle. This was identified as suillin [**1**] (4-acetoxy-3-geranylgeranyl-1,2-dihydroxybenzene), recently isolated from Boletes of the genus *Suillus*, by comparison of ms, ir, and ^1H and ^{13}C nmr with the reported data (1). No biological data have been reported for this compound, which is the main phenolic constituent of *S. granulatus*.

Suillin was tested in vitro against three cell line systems, and the following results were obtained: KB cells (human rhinopharynx cancer), ID_{50} = 0.69 μg/ml: P-388 cells (murine leukemia), ID_{50} = 0.85 μg/ml; NSCLC-N6 (human bronchopulmonary carcinoma), ID_{50} = 1.02 μg/ml. The compound was also tested in vivo for antitumor activity, and significant values were obtained against P-388 murine ascite leukemia: T/C = 151% at 5 mg/kg and 160% at 10 mg/kg.

The peracetate **2** was prepared and subjected to assays against KB cells. An ID_{50} = 1.8 μg/ml was obtained; this result shows that, at least in vitro, activity is not strongly inhibited by a complete acetylation of phenol groups.

EXPERIMENTAL

ISOLATION.—Fruit bodies of *S. granulatus* were collected on the slopes of Mt. Etna, freeze-dried, and ground. Powdered material (280 g) was extracted 3 times with CH_2Cl_2, and the filtered solution taken to dryness to give 5.8 g of crude extract. Chromatography on Sephadex LH-20 eluted with *n*-hexane–CH_2Cl_2 (1:4) and Me_2CO-CH_2Cl_2 (1:2 and 4:1) gave fractions (20 ml) which were pooled on the basis of their tlc



1 $R^1=R^2=H$, $R^3=Ac$
2 $R^1=R^2=R^3=Ac$

profile (ceric sulfate, 10% in 2 N H_2SO_4, and Fast Red salt B, 0.5% in H_2O, as spray reagents) and subjected to assays against KB tumor cells and *S. aureus* ATCC 25923 (6-mm paper disc-agar diffusion assay). Significant cytotoxic activity was found in fractions 82–94 (ID_{50} = 4.2 μg/ml), which were rechromatographed on acetylpolyamide [*n*-hexane–$CHCl_3$ (1:1) as the eluent] to give 200 mg of a product which was subjected to spectral analysis and identified as suillin [**1**] by comparison of ms, ir, ^1H nmr (250 MHz, $CDCl_3$), and ^{13}C nmr (62.9 MHz, $CDCl_3$) data with those reported in the literature (1).

ACETYLATION OF SUILLIN.—A solution of **1** (20 mg) in pyridine (0.5 ml) and Ac_2O (1 ml) was stirred at room temperature for 5 h. Conventional workup and flash chromatography [Si gel, Et_2O–*n*-hexane (3:7)] of the crude product gave the triacetate **2** (3-geranylgeranyl-1,2,4-triacetoxybenzene): uv (EtOH) λ max (ε) 262 nm (423); ir ν max 2970, 2930, 2860, 1780, 1485, 1450, 1375, 1200, 905 cm^{-1}; ^1H nmr (250 MHz, $CDCl_3$) δ 1.58 (9H, s), 1.68 (3H, s), 1.71 (3H, s), 2.03 (12H, m), 2.26 (3H, s), 2.28 (6H, s), 3.20 (2H, d, J = 7 Hz), 5.01 (1H, t, J = 7 Hz), 5.08 (3H, m), 6.98 and 7.08 (2H, AB system, J = 9 Hz); ^{13}C nmr (62.9 MHz, $CDCl_3$) ppm 167.5, 167.9, 168.7 (s, 3 × *C*OMe), 20.1, 20.5, 20.7 (q, 3 × COCH$_3$), 140.2, 141.2 (s, C-1 and C-2), 128.3 (s, C-3), 146.7 (s, C-4), 120.2, 120.4 (d, C-5 and C-6), 24.2 (t, Ar-CH_2), 26.4, 26.5, 26.6 (t, 3 × CH_2-CH=), 39.5, 39.6 × 2 (t, 3 × CH_2-C=), 120.7 (d, ArCH$_2$-*C*H=), 123.8, 124.1, 124.3 (d, *C*H=C-Me), 131.0, 134.7, 135.0, 136.1 (s, 4 × =*C*-Me), 15.9 × 2, 16.1, 17.6, 25.6 (q, 4 × CH_3-C=); ms *m/z* $[M]^+$ 524 (15), 509 (1), $[M - CH_2CO]^+$ 482 (3), 455 (18), 413 (10), 371 (5), 304 (6), 262 (11), 259 (8), 236 (16), 223 (26), 194 (18), 181 (41), 139 (42), 69 (100).

ASSAYS.—The ID_{50} cytotoxicity values using KB (2), P-388 (2), and NSCLC-N6 (3) cell lines represent the concentrations of the product required to reduce cell growth to one-half that of the control.

The in vivo tests were performed on murine ascite leukemia P-388 (2); suillin was injected ip on days 1, 5, and 9. Activity, reported as T/C (ratio of the mean survival time of the test group to that in controls, expressed as a percent), was 151% at 5 mg/kg and 160% at 10 mg/kg. Signs of toxicity were observed at doses higher than 10 mg/kg.

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