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SYNTHESIS OF GRIFOLIN, AN ANTIBIOTIC FROM A *BASIDIOMYCETE*

M. ISOBE and T. GOTO

Department of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya, Japan

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Abstract—Grifolin, 2-*trans,trans*-farnesyl-5-methylresorcinol (I), was synthesized from orcinol and farnesol.

KUBO and Mizuno[1] found antibiotic activity in *Grifola confluens* (Japanese name, Shiromaitake), from which Hirata and Nakanishi[2] isolated a white crystalline antibiotic and named it grifolin. The structure of grifolin was assigned by means of NMR spectra as 2-*trans,trans*-farnesyl-5-methylresorcinol (I).* This paper describes a synthesis of this antibiotic.

Orcinol bis-tetrahydropyranyl ether (II) obtained from orcinol and dihydropyran was converted to its 2-lithio compound by treatment with n-butyllithium in ether.

This lithio compound was condensed with farnesyl bromide (containing ca. 70% *trans,trans*-isomer) to give grifolin bis-tetrahydropyranyl ether (V). As the ether could not be purified by vacuum distillation, it was immediately hydrolysed with oxalic acid into crude grifolin, which was converted to its di-*p*-nitrobenzoate. Several recrystallizations of the product from n-hexane gave pure grifolin di-*p*-nitrobenzoate (IV), which was identical in all respects with that obtained from natural grifolin. Alkaline hydrolysis of the benzoate gave the antibiotic identical with natural grifolin (I) (Fig. 1).

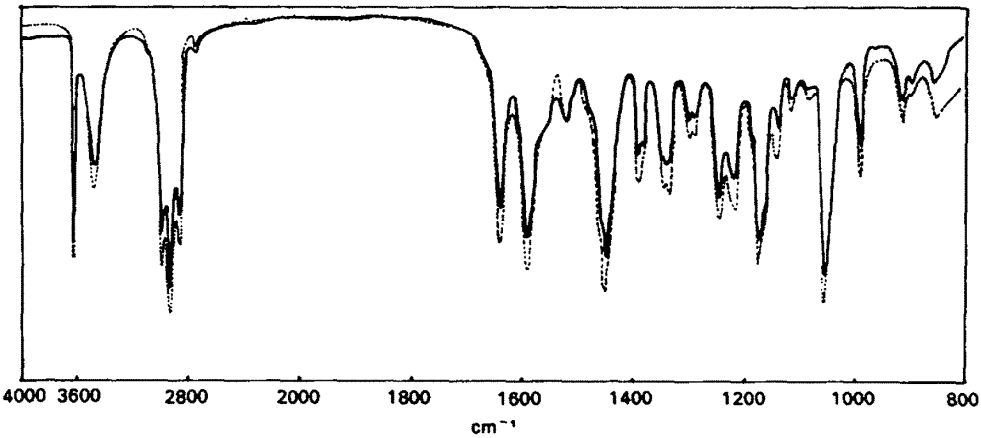


FIG. 1. The IR spectrum of grifolin (I).

——— synthetic
..... natural (in CCl_4, 0·5 mm).

* Correction: CCl_4, instead of $CDCl_3$, was actually used for the measurements of NMR spectra of grifolin and its di-*p*-nitrobenzoate reported.[3]

Introduction of the farnesyl side chain at 2-position and not at 4-position of the resorcinol moiety was demonstrated by a model reaction between lithio derivative of resorcinol bistetrahydropyranyl ether (VI) and allyl bromide. Thus, the reaction product, 2-allylresorcinol bis-tetrahydropyranyl ether (VII), was hydrolysed with oxalic acid to give 2-allylresorcinol, which was then converted into its crystalline

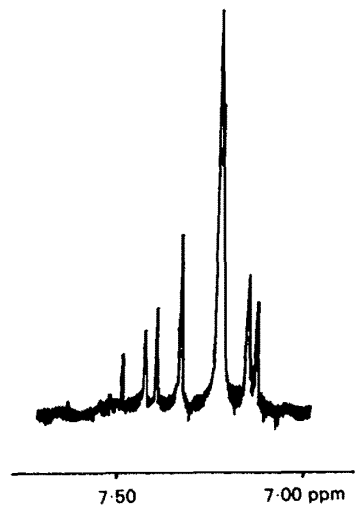
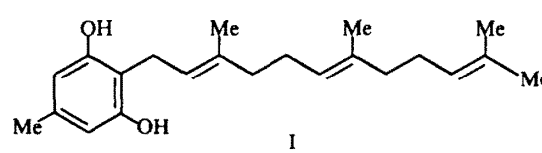
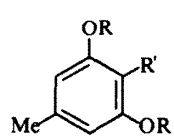


FIG. 2. The NMR spectrum of 2-allylresorcinol di-*p*-nitrobenzoate (δ-value, 100 Mc, in $CDCl_3$).

di-*p*-nitrobenzoate (VIII), m.p. 164–165°. This compound showed a typical AB_2 type signals (J = 8·4 c/s) of aromatic protons in its 100 Mc NMR spectrum (Fig. 2), indicating the presence of the allyl group at 2-position of the resorcinol moiety.



I



II: R = tetrahydropyranyl
R' = H (Li)
IV: R = *p*-nitrobenzoyl
R' = farnesyl

EXPERIMENTAL

M.ps were determined on a micro hot stage and uncorrected. The following spectrometers were used for spectral measurements: (UV) JASCO UV-175; (IR) JASCO DS-402G and IR-E; (NMR) Varian A-60A and HA-100.

Orcinol bis-tetrahydropyranyl ether (II). Treatment of anhyd orcinol with 2 moles of dihydropyran in the presence of acid catalyst by a method similar to that of Parham *et al.*[4] gave II (b.p. 160°/0·63 mmHg), in 82·6% yield; $\nu_{(neat)}$ 2920, 2855, 1595, 1200, 1150 (b), 1125, 1105–1110, 1020–1040 cm^{-1}. (Found: C, 70·17; H, 8·43. $C_{17}H_{24}O_4$ requires: C, 69·83; H, 8·27%.)

Farnesyl bromide (III). Commercial farnesol (Ardrich Co.), which contains not less than 70% of *trans, trans*-farnesol (analyzed by means of GLC[5]), was converted to farnesyl bromide in accordance with Karrer and Helfenstein's method.[6] Vacuum distillation gave a distillate at 115–127°/1·1–3·0 mmHg, which was used for the next step.

Grifolin di-p-nitrobenzoate (IV). According to Gilman's method[7] n-BuLi was prepared from n-BuBr and metallic Li. To the ether soln of 0·034 mole of n-BuLi under N_2 was added a soln of 9·8 g (0·034 mole) II in 30 ml abs ether. Being stirred for 1 hr at room temp, this soln was allowed to stand overnight for complete conversion of II into its lithio derivative. Farnesyl bromide (10 g) was added to the soln containing the lithio derivative, followed by 50 ml xylene. The mixture was heated slowly in an oil bath with removal of ether and then kept at 80–90° under reflux for 3 hr with stirring. During this time white solid (LiBr) precipitated, which indicated the proceeding of the reaction. After cooling, the reaction mixture was extracted with 100 ml ether, and the ether extract was washed (5% NaOH, 2% $NaHCO_3$, H_2O), dried (Na_2SO_4) and evaporated under vacuum to give 17 g crude V as a viscous oil (TLC shows the presence of V and hydrocarbons). A soln of the crude V (5·0 g) in 30 ml MeOH was treated with 5% aqueous oxalic acid (10 ml) at 50° for 2 hr. The reaction mixture was extracted with ether and the extract was washed with water, dried (Na_2SO_4) and evaporated under vacuum to afford 3·8 g crude I as an oil. It was dissolved in 20 ml pyridine and the soln was treated with 5·2 g *p*-nitrobenzoyl chloride. The excess acid chloride was decomposed with ice water, and the soln was extracted with ether as usual. Several recrystallizations from n-hexane gave white needles, m.p. 56–58°, which was identical with grifolin di-*p*-nitrobenzoate (m.p. 62°[2]) obtained from natural grifolin [IR, NMR, TLC (benzene, R_f = 0·50), mixed m.p.]. (Found: C, 69·12; H, 6·41; N, 4·51. $C_{36}H_{38}N_2O_8$ requires: C, 68·99; H, 6·11; N, 4·47%.)

Grifolin (I). The benzoate IV (0·5 g) was dissolved in 50% MeOH (6 ml) and stirred under N_2 with 10% KOH (3 ml) for 1·5 hr. The reaction mixture was neutralized with solid CO_2, concentrated under vacuum to remove MeOH, and extracted with ether. The ether layer was dried (Na_2SO_4) and evaporated, and the residual reddish brown oil (0·27 g) was chromatographed on silica gel (Wako Co.; 7·5 g in 4 cm dia column; solvent: benzene), and then crystallized from pet. ether (b.p. 30–50°). Several recrystallizations gave white needles m.p. 40°, which was identical with natural grifolin (m.p. 40°,[2] 43°[3]). [UV, IR (Fig. 1), NMR, TLC (benzene, R_f = 0·25), mixed m.p.]. λ_{max}^{MeOH} 281 mμ (ε = 855), 272·5 mμ (ε = 926). (Found: C, 80·66; H, 9·85. $C_{22}H_{32}O_2$ requires: C, 80·44; H, 9·83%.)

Resorcinol bis-tetrahydropyranyl ether (VI) prepared by the method of Parham *et al.*[4] b.p. 161–166°/1·5 mmHg.

2-Allylresorcinol bis-tetrahydropyranyl ether (VII). Compound VI (14·4 g) in 20 ml abs ether was stirred under N_2 with an ether soln containing 0·055 mole n-BuLi for 1 hr at room temp and the reaction mixture was allowed to stand overnight. This soln was then treated with allyl bromide (9·1 g) in 50 ml xylene and the mixture was refluxed in an oil bath at 70–80° for 2 hr (a part of ether was distilled off). After cooling, the reaction mixture was extracted with ether and the extract was washed (5% NaOH, 2% $NaHCO_3$, water), dried (Na_2SO_4) and evaporated to dryness. The residual oil was distilled under vacuum to give VII as an oily material (11·0 g; b.p. 165–180°/1·5–2 mmHg); $\nu_{(neat)}$ 2925, 2850, 1590, 1465, 1355, 1250, 1125, 1020, 905 cm^{-1}. (Found: C, 71·96; H, 8·75. $C_{19}H_{26}O_4$ requires: C, 71·67; H, 8·23%.)

2-Allylresorcinol di-p-nitrobenzoate (VIII). A soln of VII (2·0 g) in 50 ml 90% aqueous MeOH was stirred with 5% oxalic acid (5 ml) for 1 hr, and the soln was extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated under vacuum to give 0·85 g crude 2-allylresorcinol. It was dissolved in 20 ml pyridine and treated with *p*-nitrobenzoyl chloride (3·2 g). The reaction mixture was extracted with ether as usual to give, after recrystallizations from n-hexane, 2-allylresorcinol di-*p*-nitrobenzoate as colorless needles, m.p. 164–165°; ν^{KBr} 1740, 1535, 1350, 1255, 1200, 1075, 1010, 710 cm^{-1}; NMR (δ, in $CDCl_3$) 3·34 d-t (2H, J = 6; 1·5 c/s), ca. 4·81 m (1H), ca. 4·93 m (1H), 5·80 m (1H), 7·20 (2H) and 7·41 (1H) (AB_2 type; J = 8·4 c/s), 8·36 s (8H). (Found: C, 61·74; H, 3·59; N, 6·02. $C_{23}H_{16}N_2O_8$ requires: C, 61·61; H, 3·60; N, 6·25%.)

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REFERENCES

[1] H. Kubo and T. Mizuno, *Botan. Mag. Japan* **61**, 64 (1948).
[2] Y. Hirata and K. Nakanishi, *J. Biol. Chem.* **184**, 135 (1949).

[3] T. Goto, H. Kakisawa and Y. Hirata, *Tetrahedron* **19**, 2079 (1963).
[4] W. E. Parham and E. L. Anderson, *J. Am. Chem. Soc.* **70**, 4187 (1948).
[5] R. B. Bates, D. M. Gale and B. J. Gruner, *J. Org. Chem.* **28**, 1086 (1963); we thank Mr. I. Oguni for GLC analysis.
[6] P. Karrer and A. Helfenstein, *Helv. Chim. Acta* **14**, 78 (1931).
[7] H. Gilman, *Org. Reactions* **8**, 258 (1954).