to 25 mg/kg of the parent testosterone) for 8 days, while testosterone (I) was administered similarly at a dose of 25 mg/kg body weight/day as a reference standard. On the ninth day, the rats were sacrificed by decapitation, and the seminal vesicles and levator ani muscles were removed and weighed. The results were analysed statistically by the Mann–Whitney U test for the significance of the increases in the weights of the seminal vesicles and levator ani muscles with respect to the castrate controls (Table I).

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The Isolation of Secalonic Acid A from *Aspergillus ochraceus* cultured on Rice

Mikio Yamazaki, Yukio Maebayashi and Komei Miyaki

Institute of Food Microbiology, Chiba University[1]

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We have isolated an yellow crystalline compound (YC-3) from the ethyl acetate extract of *Aspergillus ochraceus* cultured on rice during our investigation on the survey of the food borne toxigenic fungi. The producibility of this compound in *Aspergillus ochraceus* has been confirmed to be as general that all of 57 strains examined in our laboratory are proved to produce this compound.

From the ethyl acetate extract of cultured mycelium of *Aspergillus ochraceus* on rice, YC-3 can easily be crystallized by treatment with chloroform, and in general, about 600 mg of YC-3 are yielded from the mycelium cultured on 1 kg of sterilized and moistened rice. YC-3 is an yellow acidic and phenolic (positive to $FeCl_3$ test) compound, having mp 243° (acetone) and 248° ($CHCl_3$). Molecular formula, $C_{32}H_{30}O_{14}$, has been elucidated from the results of the elementary analysis and M^+ 638 in mass spectrometry. In nuclear magnetic resonance (NMR) spectrometry, the signals for 12 protons excluding 3 protons of hydroxyls are observed. Accordingly, the dimeric structure has easily been expected for YC-3. The absorption maxima, 252 mμ (ε 17200) and 346 mμ (ε 31000)in ultraviolet (UV) spectrum indicate that the structure of YC-3 including its chromphores should fairly be resemble to that of ergochrome derivatives.[2] From the infrared (IR) spectral data, the presence of carboxyl ester, 1726, 1155, 1088 cm^{-1}, hydroxyl, 3480 cm^{-1}, and $-\overset{\overset{O}{\|}}{C}-C=\overset{\overset{O\cdots\cdots OH}{|}}{C}-$ 1608 cm^{-1} may be assumed. In a deutero-pyridine solution, the NMR spectral signals are obtained as follows: δ ppm from TMS, 1.26 (3H, d, J=6.0 cps) CH_3–CH$<$, 2.2—3.0 (3H, m) –CH_2–CH–, 3.56 (3H, s) OCH_3, 4.16 (1H, d, J=10.5 cps) $>$CH–O–, 6.70, 7.59 (1H, d, J=8.5 cps) two aromatic H in *ortho*-corelation.

Acetylation of YC-3 by reflux with acetic anhydride and sodium acetate afforded white amorphous acetate having mp 198°. In NMR spectrum of YC-3 acetate, four peaks at δ ppm 1.81 (3H, s), 1.91 (3H, s), 1.99 (3H, s) and 2.42 (3H, s) have clearly been observed. According to this fact, it has been initially assumed that the four acetyl groups are introduced to form the tetraacetate (octaacetate if one proposes the dimeric structure to YC-3). However,

1) Location: *3–9–1 Izumicho, Narashino, Chiba.*
2) B. Franck, G. Baumann and U. Ohnsorge, *Tetrahedron Letters*, **1965**, 2031 and ref. cited therein.

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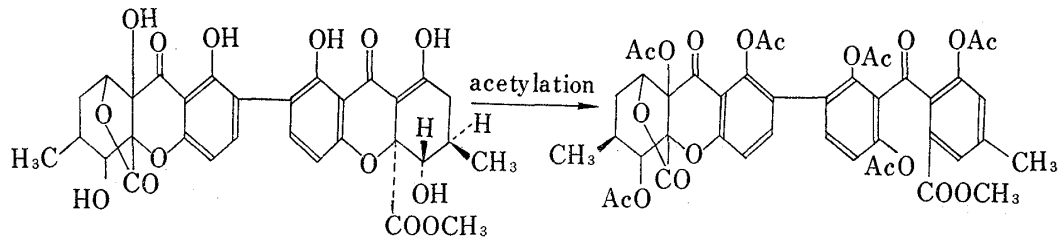
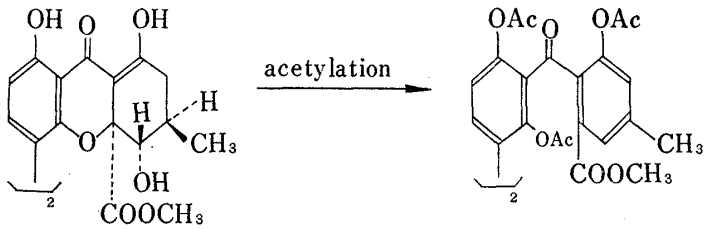
since the molecular formula, $C_{44}H_{38}O_{18}$, has been elucidated from the elementary analysis and M^+ 854 in mass spectrometry, the assumption such as the four acetyls (eight in dimeric structure) are present in the molecule should be quite incompatible. On the acetylation of ergochrysin A, Aberhart, *et al.*[3] have reported that the aceytlation reaction must be proceed ed as indicated below.



ergochrysin A ergochrysin A acetate

According to the fact as mentioned above, one of the four peaks in NMR spectrum of our acetate should be assumed to be a signal for the aromatic methyl which is newly fomed during acetylation. In addition, four aromatic protons at δ ppm 7.16 and 7.56 in *meta*-corelation each other are observed in NMR spectrum of the acetate. The occurence of aromatization during acetylation is therefore quite plausible.

Conclusively, all of the information on the structure of YC-3 is completely identical with that of secalonic acid A isolated first by Franck, *et al.*[2] as a component of cultured *Claviceps purpurea*. No depression of melting point of YC-3 was also observed in the mixed fusion with authentic secalonic acid A. The aromatization during acetylation procedure on YC-3 must be undertaken as shown below.



secalonic acid A (YC-3) acetate

Although YC-3 (secalonic acid A) has been detected from all of the strains of *Aspergillus ochraceus* which we examined, no one has reported so far the isolation of this compound from the genus Aspergillus including *Aspergillus ochraceus*.

The biosynthetic derivation of this type of compound from anthraquinone has been studied by Franck, *et al.*[4] with isotopic tracer technique. The coexistence of emodin with secalonic acid in *Aspergillus ochraceus* that has been confirmed simultaneously in our investigation supports the proposed biosynthetic route for this type of compound.

Although no lethal examples are observed by oral administration of 250 mg/kg to mice, whole of 8 at 100 mg/kg dose and 6 among 8 mice at 50 mg/kg dose administration of this compound have been dead by intraperitoneal injection. No toxicity of this compound to chicken embryos has been observed.

3) D.J. Aberhart, Y.S. Chen, P. de Mayo and J.B. Stothers, *Tetrahedron*, **21**, 1417 (1965).
4) B. Franck and F. Hüper, *Angew. Chem.*, **78**, 752 (1966).

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The antimicrobial activity of this compound for *Bacillus subtilis* and *Piricularia oryzae* has been recognized by disc assay but no striking effect to other microorganisms have obtained. Steyn[5] has reported in addition that LD 50 of secalonic acid D (an optical antipode of secalonic-acid A) for mice in intraperitoneal injection is 42 mg/kg. The investigation on the toxicity or the biological activity of this compound is now being continued and the details of the results will be discussed elsewhere.

Experimental

Microorganism——All of 57 strains of *Aspergillus ochraceus* used in our experiment were isolated from the moldy rice collected in Chiba and Miyagi prefectures in our own laboratory, and maintained on malt extract agar or Czapek agar containing 20% of sucrose.

Isolation and Properties of YC-3——The fungus was cultured stationary on sterilized rice (1 kg) for 9 days at 27° in dark, and extracted with EtOAc. The solvent was removed under the reduced pressure. The residue was resolved again in $CHCl_3$ and shaken with 10% Na_2CO_3 solution. After acidifing the alkali solution, the aqueous layer was extracted with $CHCl_3$. By concentration of the $CHCl_3$, the crude YC-3 was crystallized. Recrystallization from $CHCl_3$ afforded yellow needles (600 mg).

The physical data of YC-3 were as follows: mp 243° (from acetone) and 248° (from $CHCl_3$); UV λ_{max}^{EtOH} mμ: (ε) 346 (31000), 252 (17200). IR (KBr) cm^{-1}: 3490, 2950, 1726, 1608, 1587, 1560, 1435, 1325, 1235, 1160, 1130, 1063, 1025, 987, 905, 825. NMR δ ppm from TMS (in d-pyridin) 1.26 (6H, d, J=6.0 cps, 2×CH_3), 2.2—3.0 (6H, m), 3.56 (6H, s, 2×CH_3O), 4.16 (2H, d, J=10.5 cps, >CH–O–), 6.70 (2H, d, J=8.5 cps, aromatic H), 7.59 (2H, d, J=8.5 cps, aromatic H). Mass Spectrum *m/e*: 638 (M^+), base peak *m/e* 579 (M-59 (CO_2CH_3)). Elementary analysis. *Anal.* Calcd. far $C_{32}H_{36}O_{14}$: C, 60.19%; H, 4.74%. Found: C, 60.06%; H, 4.68%. Red-brown color was observed in $FeCl_3$ test.

Acetylation of YC-3——The mixture of YC-3 (250 mg), sodium acetate (1 g) and acetic anhydride (9 ml) was refluxed for 2 hours on a mantleheater. After cooled, the reaction mixture was poured into ice water and extracted repeatedly with ether. Evaporation of ether gave crude precipitates of the acetate, which was treated with acetone to yield white amorphous plates of YC-3 acetate (190 mg).

The physical data of YC-3 acetate were as follows: mp 198° (from acetone). UV λ_{max}^{EtOH} mμ: (ε) 290 sh. (4850). IR (KBr) cm^{-1}: 1776, 1730, 1680, 1613, 1185, 1032. NMR δ ppm from TMS (in $CDCl_3$) 1.81 (6H, s), 1.97 (6H, s), 1.99 (6H, s), 2.42 (6H, s), 3.70 (6H, s), 7.08 (2H, d, J=8 cps), 7.44 (2H, d, J=8 cps), 7.16 (2H, bs), 7.56 (2H, bs). Mass Spectrum *m/e*: 854 (M^+). Elementary analysis. *Anal.* Calcd. for $C_{44}H_{38}O_{18}$: C, 61.82%; H, 4.44%. Found: C, 62.13%; H, 4.22%.

Isolation of Emodin——On the silicagel chromatography of $CHCl_3$ filtrate after removal of YC-3 crystals, a orange band was observed. The colored fractions were gathered together and crystalized with $CHCl_3$ to yield orange red needles.

The spectral data of the pigment were as follows: mp 245—250° (from $CHCl_3$). UV λ_{max}^{EtOH} mμ: (ε) 222 (35500), 252 (18200), 265 (18650), 289 (21900), 435 (12600). IR (KBr) cm^{-1}: 3386, 1625, 1560, 1477. NMR δ ppm from TMS (in d-acetone) 2.45 (3H, s, aromatic CH_3), 6.03 (1H, d, J=2.3 cps), 7.21 (1H, d, J=2.3 cps), 7.10 (1H, d, J=1 cps), 8.52 (1H, d, J=1 cps). This orange red pigment was completely identical with an authentic sample of emodin by also a mixed fusion.

Assay Method for Toxicity and Antimicrobial Activity——YC-3 was dissolved in 5—10% $NaHCO_3$ solution, neutralized with diluted HCl and injected intraperitoneally into the male mice, ddys strain (av. body weight 27.5 g).

For chicken embryo test, the solution was diluted with distilled water as that 100 and 200 μg/egg were administered to the eggs respectively when 0.2 ml of the solution were injected. The solution was injected into yolk sac.

The antimicrobial assay was carried out by disc method. YC-3 was dissolved in ethanol to make the sample solution having the concentration as 50 and 500 μg/ml. The microorganisms used are as follows: *Staphylococcus aureus* FDA 209P, *Bacillus subtilis* pcl 219, *Eschelischia coli* NIH-J, *Pseudomonas aeruginosa*, *Mycobacterium sp* 607, *Candida albicans* 3147, *Trichophyton mentagrophytes* 640, *Piricularia oryzae* and *Xanthomonas oryzae*.

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5) P.S. Steyn, *Tetrahedron*, **26**, 51 (1970).

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