sence of 100 μg of xylose according to Dische's and modified method, $\Delta A_{510-540\ nm}$ should be 0.200 and 0.231. They are 90.5% and 104.5% of real value, respectively. The interferences of phosphorylated sugars which are real substrates, on sedoheptulose determination are in study. Pentose, hexose and heptulose are all reactive with sulfuric acid to give furfural derivatives which then react with SH containing reagents producing colored substances. Their structures are still unknown. It is interesting to note, however, that SH compounds give intense colours for pentoses in the order of their molecular weight; glutathione> cysteine>thioglycolic acid>H_2S.

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Tetrahydroauroglaucin from *Penicillium charlesii*

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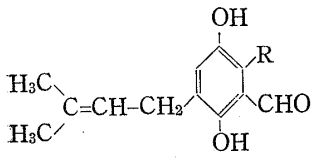
In our course of chemical examinations on the metabolites produced by toxic fungi,[2] we encountered a strain of *Penicillium charlesii* G. SMITH highly colored in the mycelium. Although the fungus had been known to metabolize tetronic acid derivatives,[3] pigment formation had not been reported. Thus chromatographic separation of the chloroform extract of the mycelium was performed to give three pigments; physcion (parietin) (I), flavoglaucin (II), and a new yellow pigment of mp 60—61° (III). The two (I and II) have been known as common metabolites of *Aspergillus glaucus* group and I has also been isolated from some other fungi.[3]

The new pigment (III) has a molecular formula, $C_{19}H_{26}O_3$ (by a high resolution mass spectrum), and shows quite similar spectral properties to II; the infrared (IR) spectrum (ν_{max}^{KBr} cm^{-1}: 3250, 2900, 1620, 1580, 1440, 1260) shows the presence of hydroxyl and hydrogen-bonded conjugated carbonyl groups and the ultraviolet (UV) spectrum (λ_{max}^{EtOH} nm(log ε): 277, 400 (3.93, 3.69)) is nearly the same but shows a slight bathochromic shift comparing to that of II (λ_{max}^{EtOH} nm(log ε): 270, 394 (3.85, 3.66)). These spectral data, the molecular formula, and coexistence with II suggested that III might be a dehydro derivative of II, *i.e.* a tetrahydro derivative of auroglaucin[3] (IV), a congener of II in *Aspergillus glaucus* group. The comparison of th nuclear magnetic resonance (NMR) spectra of II and III disclosed clearly the assumption: The signals for the dimethylallyl, bonded and non-bonded hydroxyls, ring hydrogen, and formyl groups are seen in the spectra of both compounds but the methylene signal (δ *ca.* 1.4) corresponding to five methylenes in II was reduced to that of three in III,

1) Location: *Kamiyoga-1-chome, Setagaya-ku, Tokyo.*
2) The work is in progress with the colaboration with Prof. M. Saito, Institute of Medical Sciences, University of Tokyo, and Dr. H. Kurata, this Institute, and their colaborators; *cf.* M. Saito, M. Umeda, K. Otsubo, H. Kurata, S. Udagawa, and S. Natori, *Proc. Japan Cancer Assoc.*, 27th Annual Meeting, Tokyo, 1968, p. 59.
3) S. Shibata, S. Natori, and S. Udagawa, "List of Fungal Products," University of Tokyo Press, Tokyo, 1964; W.B. Turner, "Fungal Metabolites," Academic Press, London and New York, 1971.

the benzylic methylene signal (δ 2.85) in II disappears in III and an allylic methylene signal (δ 2.30) appears instead, and a *trans*-olefinic signal (δ 6.50 (d, J=16.5 Hz), 5.95 (dt, J=16.5, 6 Hz)) newly appears in III. The bathochromic shift shown in the UV of III comparing to that of II and the chemical shifts and the coupling patterns of the olefinic protons of III indicated that the double bond must locate in the conjugation with the benzene ring; thus the structure (III) was proposed. The mass spectrum of III was also in a good accord with the formulation.



II: R=$-C_7H_5$

III: R=$-C=C-C_5H_{11}$ (H)

IV: R=$-(CH=CH)_3-CH_3$

At this stage of the work we learned that Sokolov, *et al.*[4] isolated an antibiotic, named aspergin, mp 90.5—91.5°, from an unidentified strain of *Aspergillus* sp. and proposed the same structure (without the configurational assignment of the double bond) from the spectral data and some reactions. The comparison of ours with the sample of aspergin, kindly supplied by Dr. Sokolov, showed a difference between the two by mp, IR, NMR, and thin-layer chromatography (TLC). Due to the scarcity of our sample and the mutation of the mold in production of the pigments, further work was abandoned.

Experimental

Cultivation of *P. charlesii* and Isolation of Physcion (I), Flavoglaucin (II), and Tetrahydroauroglaucin (III)——The strain NHL 6139 of *P. charlesii*, isolated from miso (soybean paste) collected at Amami Is., Kagoshima Pref.,[5] was incubated in the modified Czapek medium (containing malt extract (0.5 g/liter) and yeast extract (0.2 g/liter), 200 ml×5) at 25° for 3 weeks by surface culture. The mycelium freed from the medium was directly extracted with chloroform and the extract was passed through a column of acid-treated silica gel. The yellow-colored zone was collected and further separated by preparative layer chromatography on acid-treated silica gel plates into three bands using hexane–chloroform (4: 1) as the developer.

The first band from the top was extracted and recrystallized from hexane to yellow leaflets (III) (33 mg) of mp 60—61°. UV λ_{max}^{EtOH} nm(log ε): 277, 400 (3.93, 3.69). IR ν_{max}^{KBr} cm^{-1}: 3250, 2900, 1620, 1580, 1485, 1440, 1400, 1260, 990, 950. NMR δ (in $CDCl_3$): 0.90 (3H, t, J=5 Hz), 1.4 (6H, br.), 1.70 (3H, br. s), 1.73 (3H, br. s), 2.30 (2H, br. q, J=6 Hz), 3.30 (2H, br. d, J=7 Hz), 5.0 (1H, br.), 5.28 (1H, br. t, J=7 Hz), 5.95 (1H, dt, J=16.5, 6 Hz), 6.50 (1H, d, J=16.5 Hz), 6.96 (1H, s), 10.02 (1H, s), 11.62 (1H, s). Mass Spectrum *m/e*: 302.183 (M^+, calcd. for $C_{19}H_{26}O_3$, 302.188), 269, 247, 231, 228, 189, 175.

The second fraction was purified from $CHCl_3$ to orange needles (4 mg) of mp 206° (lit.[6] mp 204—205°). IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1630, 1565, 1480, 1390, 1370, 1325, 1272, 1230, 1165, 1105, 1035, 980. NMR δ (in $CDCl_3$): 2.40 (3H, br. s), 3.85 (3H, s), 6.50 (1H, d, J=3.5 Hz), 6.90 (1H, br. d, J=3 Hz), 7.20 (1H, d, J=3.5 Hz), 7.45 (1H, br. d, J=3 Hz), 11.83 (1H, s), 12.03 (1H, s). The direct comparison with the sample of physcion (I) by IR and TLC showed the identity.

The third band gave yellow leaflets (21 mg) of mp 99—100° (lit.[6] mp 102—104°) from $CHCl_3$. IR ν_{max}^{KBr} cm^{-1}: 3280, 2920, 1630, 1590, 1485, 1450, 1305, 1225, 1120, 935. NMR δ (in $CDCl_3$): 0.86 (3H, t, J=5 Hz), 1.3 (10H, br.), 1.70 (3H, br. s), 1.75 (3H, br. s), 2.85 (2H, t, J=7 Hz), 3.26 (2H, br. d, J=7.5 Hz), 4.4 (1H, br.), 5.20 (1H, br. t, J=7.5 Hz), 6.78 (1H, s), 10.08 (1H, s), 11.70 (1H, s). Mass Spectrum *m/e*: 304.200 (M^+, calcd. for $C_{19}H_{28}O_3$, 304.204), 249, 230, 215, 150. The comparison with the authentic sample of flavoglaucin[6] (II) by IR and TLC showed the identity.

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