CHEMISTRY LETTERS, pp. 1491-1492, 1974. Published by the Chemical Society of Japan

A NEW PHTHALIDE FROM A FUNGUS, *ALTERNARIA KIKUCHIANA*[1)]

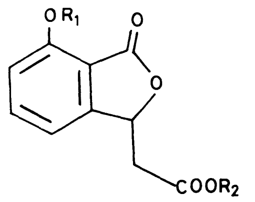
Kiyoshi KAMEDA and Mitsuo NAMIKI
Faculty of Agriculture, Nagoya University, Nagoya 464

A new phthalide was isolated as a metabolite of Alternaria kikuchiana and identified as 3-carboxymethyl-7-hydroxyphthalide (1a).

Alternaria kikuchiana is known as a parasite which causes black spot disease on Japanese pears and some of its toxic metabolites have been reported.[2)] During the purification of the host specific toxins, we have isolated a new metabolite with which is concerned in this communication.

Culture filtrates (ca. 100 1, pH 3) of the broth of *Alternaria kikuchiana* grown on potato decoction medium was extracted with ether and the acidic fractions of the ether extracts were subjected on silicic acid column chromatography and preparative TLC to give colorless crystals (1a) (7.2 mg, mp. 162°C) which were named as iso-ochracinic acid. It gave a weak blue fluorescence under uv irradiation and a purple coloration with ferric chloride. Its molecular formula was determined to be $C_{10}H_8O_5$ by high resolution mass spectrography[3)] (m/e 208.0368, calcd. for $C_{10}H_8O_5$ .0371). The UV spectrum (λ_{max}^{MeOH}nm (log ε) 234 (3.73), 300 (3.54); $\lambda_{max}^{MeOH\text{-}NaOH}$ 222, 248, 336) is very similar to that of 7-hydroxyphthalide (2a) (λ_{max}^{EtOH} 232 (3.93), 299 (3.66)).[4)] Furthermore, it is in good agreement with that of iso-ochracin (3a), which has been derived chemically from mellein by Yabuta and Sumiki[5)] and has recently been isolated as a new metabolite of *Hypoxylon coccineum* (λ_{max}^{MeOH} 234 (3.86), 300 (3.66); $\lambda_{max}^{MeOH\text{-}NaOH}$ 221, 247.5, 336)).[6)] These facts suggest the presence of 7-hydroxyphthalide skelton in (1a). Acetylation and methylation of (1a) afforded monoacetate (1b) $C_{12}H_{10}O_6$ (m/e 250.0474, δ^{CDCl_3} 2.40) and dimethylate (1c) $C_{12}H_{12}O_5$[7)] (m/e 236.0686), indicating (1a) possesses one phenolic and one carboxylic groups, respectively. Consequently, five oxygen functions in (1a) are all assigned. Since the UV (λ_{max}^{MeOH} 236 (3.67), 298 (3.45)) and NMR spectra (especially on the aromatic protons, δ^{CDCl_3} 6.98,d,J=8, 7.03,d,J=8, 7.64,t,J=8) of (1c) are compatible with those of 7-methoxyphthalide (2b) (λ_{max}^{MeOH} 235 (3.81), 297 (3.63); δ^{CDCl_3} 6.97,d,J=8, 7.04,d,J=8, 7.66,t,J=8)[8)], OMe group in (1c) should be located at C-7. On the other hand, the NMR spectrum ($(CD_3)_2CO\text{-}CDCl_3$) of (1a) shows three adjacent tri-substituted benzene protons (δ 6.97,d,J=8, 7.12,d,J=8, 7.60,t,J=8), exchangeable protons (δ 5.5-6.0 br.), methylene and methine protons which are adjacent to each other (δ 2.95,d,J=7, 5.91,t,J=7). The fact that the chemical shifts of methylene and methine protons are considerably low suggests the presence of electron withdrawing groups in their neighbourhood, that is, methine proton may be vicinal both to benzene ring and oxygen atom and methylene protons may be vicinal to benzylic methine and carboxylic group. Therefore, the remaining substituent (CH_2COOH) must be attached to C-3 on the phthalide ring.

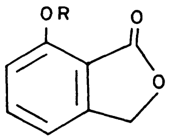
In the mass spectrum of (1a), the presence of the fragment ions $C_9H_6O_3$ and $C_8H_5O_3$ corresponding to loss of HCOOH and CH_2COOH from the parent ion, also strongly support these assignments mentioned above. Thus, the structure of iso-ochracinic acid was established as 3-carboxymethyl-7-hydroxyphthalide (1a). The biogenesis of iso-ochracinic acid is of interest because the phthalide derivatives substituted at C-3 are rare as fungal metabolites.[9] Synthesis of iso-ochracinic acid as well as the survay of its biological activities is undertaken.



1a R1 = R2 = H

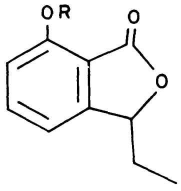
1b R1 = Ac, R2 = H

1c R1 = R2 = Me



2a R = H

2b R = Me



3a R = H

3b R = Me

Acknowledgements: we are grateful to professor J. D. Bu'Lock and Dr. P. R. Page for the gifts of iso-ochracin and O-methyl iso-ochracin.

References and Notes

1) Studies on the Metabolites of Alternaria kikuchiana, part V
2) I. Hiroe, S. Nishimura and M. Sato, Bull. of Fac. of Agric., Tottori University, 11, 291 (1958)
N. Sugiyama, C. Kashima, M. Yamamoto, T. Sugaya and R. Mohri, Bull. Chem. Soc. Japan, 39, 1573, 2470 (1966)
3) Mass spectra were measured with JMS-01SG spectrometer (Japan Electric Optics Lab.)
4) J. Blair, J. J. Brown and G. T. Newbold, J. C. S., 1955, 708
5) T. Yabuta, and Y. Sumiki, J. Agric. Chem. Soc. Japan, 10, 703 (1934)
cf. J. Blair and G. T. Newbold, J. C. S., 1955, 2871
6) Private communication from prof. J. D. Bu'Lock
7) Repeated crystalization failed to remain an oil
8) Prepared from 6-methoxyphthalaldehydic acid with sodium borohydlide
9) W. B. Tuner, "Fungal metabolites" Academic Press, London and Newyork, 1971, P 118

(Received August 30, 1974)