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METABOLITES OF *CERCOSPORA*. TAIWAPYRONE, AN α-PYRONE OF UNUSUAL STRUCTURE FROM *CERCOSPORA TAIWANENSIS*

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Key Word Index—*Cercospora taiwanensis*; Ascomycetes; (+)-mellein; 3*S*,4*S*-4-hydroxymellein; α-pyrones; structural determination.

Abstract—(+)-Mellein (**1**), cis-3*S*,4*S*-4-hydroxymellein (**3**), and taiwapyrone (**4**), a new α-pyrone, have been isolated from the mycelium of *Cercospora taiwanensis*, grown on potato-agar. The structure and absolute configuration of (**3**) and (**4**) have been elucidated.

INTRODUCTION

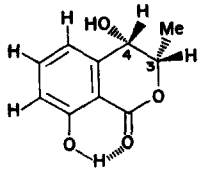
The fungi of the genus *Cercospora* are well known as pathogens of a number of crops, such as sugarbeets and soyabeans. However, little work has been done on the secondary metabolites produced by these micro-organisms. The only metabolite, which has interested several groups [1] is cercosporin, the phytotoxic perilenequinone produced by *C. beticola*, *C. kikuchii* and other strains, the structure [1,2,3] and the stereochemical features [4] of which have been elucidated. We have also recently reported on the structure of three new metabolites from *C. ligustrina* Boerema [5]. The present work concerns the metabolites produced by *C. taiwanensis*.

RESULTS AND DISCUSSION

The fungus was grown on potato-agar medium, and the metabolites extracted with ethyl acetate and purified by chromatography. Four main products (**1**–**4**) were obtained as pure compounds.

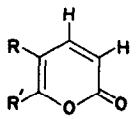
Spectroscopic data (IR, UV, NMR and MS) indicate that metabolite (**1**) is (+)-mellein, a well known substance isolated from *Aspergillus melleus* and from other imperfect molds [6]. The metabolite (**2**) was shown to be *p*-hydroxybenzaldehyde by comparison with an authentic sample.

Compound (**3**) was shown to be *cis*-4-hydroxymellein previously isolated by Turner *et al* [7] from *Lasiodiplodia theobromae*, and its absolute configuration was determined as follows. Treatment of (**3**) with PBr_3, and direct hydrogenation of the bromide with 5% Pd/C as a catalyst gave (+)-mellein (**1**), whose absolute configuration is known to be 3*S* [9]. Therefore, as H_3 and H_4 in (**3**) are *cis*, the absolute configuration of (**3**) must be 3*S*,4*S*. The same conclusion came from application of the Horeau method [10] to (**3**). Assuming that the steric requirements are the following, –CH(Me)–O– > C_6H_5 > H, the absolute configuration at C_4 is *S* as shown in formula (**3**). *Trans*-4-hydroxymellein of unknown configuration [8a] and 4-hydroxymellein of unassigned stereochemistry [8b] have also been isolated from *Apiospora camptospora* and *Aspergillus ochraceus* respectively.



(**3**)

The fourth metabolite (**4**), that we propose to call taiwapyrone, is an oil. Both UV (λ_{max} 298 nm) and IR spectra (3400 cm^{-1}, OH, 1710 cm^{-1}, conj. lactone) are consistent with the presence of an α-pyrone ring. The MS of the diacetate of (**4**) shows the presence of two OH groups: M^+ *m/e* = 282, *m/e* = 222 ($M^+ - 60$), *m/e* = 180 ($M^+ - 42$). The PMR spectrum of (**4**) shows a Me on an aliphatic chain as a doublet, a $-CH_2-CH_2-$ group, a $-CH_2-OH$ (not coupled with other protons) shifted by acetylation (J_{gem} = 14 Hz), a –CHOH– (shifted by acetylation) as a triplet at δ 4·94 and two protons on a double bond in a ring (J_{AB} = 10 Hz). The ^{13}C NMR of (**4**) shows signals corresponding to 10 carbons: three of them show shifts characteristic of a $Me-CH_2-CH_2-$ [11], a C=O (singlet), a –CHO– (doublet), a $-CH_2-O-$ (triplet) and four aromatic carbons (two doublets and two singlets), (Table 1). Combination of all these data gives for (**4**) a partial structure of a disubstituted α-pyrone; the position of the two hydrogens on the α-pyrone ring is established by the value of 10 Hz of the coupling constant in the PMR spectrum indicating that they are adjacent and in position 3 and 4 [12], hence a partial structure for (**4**) is (**5a,b**).



(**5a**) R = $-CH_2-OH$; R′ = $-CHOH-CH_2-CH_2-Me$
(**5b**) R = $-CHOH-CH_2-CH_2-Me$; R′ = $-CH_2-OH$

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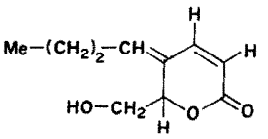
Table 1. ^{13}C NMR ($CDCl_3$) for taiwapyrone (4) and its diacetate (shifts in ppm; s = singlet, d = doublet, t = triplet, q = quartet)

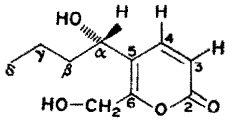
	Taiwapyrone	Taiwapyrone diacetate
C_2	162·171 (*s*)	160·843 (*s*)
C_3	115·980 (*d*)	117·360 (*d*)
C_4	144·466 (*d*)	142·413 (*d*)
C_5	120·462 (*s*)	117·809 (*s*)
C_6	158·653 (*s*)	155·837 (*s*)
Cα	67·746 (*d*)	69·657 (*d*)
Cβ	38·997 (*t*)	36·416 (*t*)
Cγ	18·879 (*t*)	18·717 (*t*)
Cδ	13·885 (*q*)	13·703 (*q*)
$-CH_2-O-$	58·600 (*t*)	59·847 (*t*)
2 CO–Me	—	170·311 (*s*)
2 $\underline{CH_3}$–CO	—	20.918–20·612 (*q*)

In order to establish the exact position of the two substituents we reacted (4) with $LiAlH_4$ and obtained the product (6). Its IR spectrum shows an absorption at 1730 cm^{-1} clearly indicating that the pyrone carbonyl is still present. The MS of the acetate of (6) shows a M^+ at $m/e = 224$ and a fragment at $m/e = 182$ ($M^+ - 42$) indicating that only one OH group is now present in (6). In the PMR of (6) the $-CH_2-OH$ group appears to be adjacent to a new $-CH-O-$, the protons H_3 and H_4 are still present on the same double bond and a new vinylic hydrogen at δ 5 is coupled with a $-CH_2-$ of the chain. The chemical shift of the new proton at δ 5·3 [13] requires that it must be on a carbon bearing an oxygen atom i.e. on C_6. Therefore the $-CH_2-OH$ group must be on C_6 and it follows that the only position for the chain is on C_5. These data can be explained on the basis of a reduction of the 5,6 double bond by $LiAlH_4$ and elimination of H_2O between H_5 and the $-CHOH-$ group of the chain. Thus (6) must have the formula



(6)

and the structure of taiwapyrone is (4)



(4)

The absolute configuration of the α-carbon of the chain was again established as *S* by the Horeau method [10], assuming that the heterocyclic group with a bulky $\alpha-CH_2-OH$ group has greater steric requirements that the propyl chain.

A number of α-pyrones with aliphatic side chains, e.g. pestalotin [14], 6-pentylpyrone [15] and related derivatives [16] have been recently isolated from fungi. It is tempting to suggest that taiwapyrone (4) is biogenetically derived from a pentaketide unit. However a cyclization of this unit, followed by oxidation and ring closure, seems necessary to account for the unusual structure of taiwapyrone (4).

EXPERIMENTAL

Mp's are uncorrected. UV spectra were measured in 95% EtOH. NMR spectra were recorded at 100 MHz and, chemical shifts are in ppm (δ), from TMS as internal standard. Column chromatography and TLC were performed with Si gel. Where not otherwise indicated the purity of the products was checked by TLC and NMR and deemed sufficient for the purposes of structural elucidation.

Isolation and purification of the metabolites. A strain of *Cercospora taiwanensis* 139·35, obtained from Centraalbureau voor Schimmelcultures, Baarn, was grown on potato-agar medium in Roux flasks at room temp. for 3 wks. Mycelia were crushed and extracted 2× with EtOAc (100 ml per flask) until the extract was colourless. Extract was dried and evaporated to give a brown mixture of crude pigments (1·63 g from 40 flasks). This material was adsorbed on the top of a chromatographic column and eluted with a mixture of hexane and EtOAc. The four metabolites: (1) mellein (400 mg), (2) *p*-hydroxybenzaldehyde (10 mg), (3) 4-hydroxymellein (50 mg) and (4) taiwapyrone (140 mg) were obtained with hexane–EtOAc eluates of 4:1, 2:1, 2:1, 1:1 respectively.

(+)-mellein. White needles mp 120–122°, $[\alpha]_D^{20} = +83·5°$ (c = 0·6, MeOH).

Cis-3S,4S-4-hydroxymellein. White solid mp 118–119°, $[\alpha]_D^{20} = +37·4°$ (c = 0·33, MeOH). λ_{max} 246 nm and 314 nm (ε 5000,3700). γ_{max} (Nujol) cm^{-1} 3300 (OH), 1680 (C=O). PMR($CDCl_3$) Me (δ 1·57), aliphatic OH (δ 2·19), H_3 (δ 4·70), H_4 (δ 4·75), $J_{3,4}$ 2 Hz, a group of three adjacent aromatic hydrogens H_5 (δ 6·9), H_6 (δ 7·54), H_7 (δ 7·1), a chel. OH (δ 10·98).

Cis-3S,4S-4-hydroxymellein diacetate. 50 mg of (3) in 0·5 ml of dry C_5H_5N and 1 ml Ac_2O were left for 6 hr at room temp. Dilution with H_2O, extraction with EtOAc and PLC gave the diacetate as a solid, mp 80–82°.

5-[1-(1S)-hydroxybutyl]-6-(hydroxymethyl)-2H-pyran-2-one (4). Oil. Mass 198, 156, 138. λ_{max} 298 nm (ε 5750). γ_{max} (neat) cm^{-1} 3400 (OH), 1710 (conj. CO). PMR($CDCl_3$) Me (δ 0·97), $-CH_2-CH_2-$ (δ 1·16–2·0), $-CH_2-O-$ (δ 4·40–4·58), J_{gem} 14 Hz, $-CHO-$ as a triplet (δ 4·94), H_3 (δ 6·3), H_4 (δ 7·54), $J_{3,4}$ 10 Hz. $[\alpha]_D^{20} = -48·5°$ (c = 0·33, MeOH).

5-[1-(1S)-hydroxybutyl]-6-(hydroxymethyl)-2H-pyran-2-one diacetate. 50 mg of (4) in 0·5 ml of dry C_5H_5N and 1 ml Ac_2O were left for 6 hr at room temp. The diacetate was obtained as an oil. Mass 282. λ_{max} 293 nm (ε 5400). γ_{max} (neat) cm^{-1} 1745 (CO–Me and conj. CO). PMR($CDCl_3$) Me (δ 1·00), $-CH_2-CH_2-$ (δ 1·2–2·05), two $-O-COMe$ (δ 2·06–2·12), $-CH_2-O-$ (δ 4·94–5·04), J_{gem} 14 Hz, $-CH-O-$ coupled with a $-CH_2-$ (δ 5·71). $J_{AB} + J_{AC}$ 14 Hz, H_3 (δ 6·37), H_4 (δ 7·41), $J_{3,4}$ 10 Hz.

5-butylidene-6-hydroxymethyl-5,6-dihydro-2H-pyran-2-one (6). A small excess of $LiAlH_4$ was added, with stirring, to 50 mg taiwapyrone (4). Dilution with H_2O, acidification, extraction with EtOAc and chromatography yielded (6) as an oil. λ_{max} 271 nm (ε 8250). γ_{max} (neat) cm^{-1} 3400 (OH), 1700 (conj. CO). PMR(C_6D_6) Me (δ 0·76), $-CH_2-CH_2-$ (δ 1–1·8), $-CH_2-O-$ (δ 3·58–3·68), J_{gem} 14 Hz, $-HC=$ (δ 5), H_6 (δ 5·3), H_3 (δ 5·64), H_4 (δ 6·06), $J_{3,4}$ 10 Hz.

5-butylidene-6-acetoxymethyl-5,6-dihydro-2H-pyran-2-one. Acetylation as described above gave (6) acetate as an oil. Mass 224, 182 ($M^+ - 42$). λ_{max} 267 nm (ε 1290). γ_{max} (neat) cm^{-1} 1730 (CO–Me and conj. CO). PMR(C_6D_6) Me (δ 0·93), $-CH_2-CH_2-$ (δ 1·1–1·6), $-CH_2-O-$ as a doublet (δ 4·22), $-CH=$ as a triplet (δ 4·96), $-CH-O-$ as a triplet (δ 5·23), H_3 (δ 5·63), H_4 (δ 6·04), $J_{3,4}$ 10 Hz.

Treatment of (3) with 2-phenylbutyric anhydride. 60 mg of 2-phenylbutyric anhydride were added to 36 mg of (3) dissolved in 1 ml dry C_5H_5N. (−)-2-phenylbutyric acid $[\alpha]_D^{20} = -11·1°$ (c = 1·5, pyridine) was obtained by working up the reaction mixture according to [10].

Treatment of taiwapyrone (4) with 2-phenylbutyric anhydride. 135 mg of 2-phenylbutyric anhydride were added to 43 mg of taiwapyrone (4) dissolved in 1 ml dry C_5H_5N, made up as

above gave (−)-2-phenylbutyric acid $[\alpha]_D^{20} = -12{\cdot}3°$ ($c = 1{\cdot}3$, pyridine).

Hydrogenation of 4-hydroxymellein (**3**). To 10 mg (**3**) dissolved in dry C_6H_6 was added 1 ml of PBr_3 at room temp. over a period of 30 min. Dilution with H_2O, extraction with EtOAc and PLC yielded a compound which was directly hydrogenated with 5% Pd/C as a catalyst in HCl–MeOH. By PLC (+)-mellein was obtained $[\alpha]_D^{20} = +68{\cdot}5°$ ($c = 0{\cdot}66$, MeOH).

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REFERENCES

1. Kuyama, S. and Tamura, T. (1957) *J. Am. Chem. Soc.* **79,** 5725; Kuyama, S. (1962) *J. Org. Chem.* **27,** 939.
2. Balis, C. and Payne, M. G. (1971) *Phytopathol.* **61,** 1477; Mumma, R. O., Lukezic, F. L. and Kelly, M. G. (1973) *Phytochemistry* **12,** 917; Kanthamani Venkataramani (1967) *Phytopathol. Z.* **58,** 379.
3. Lousberg, R. J. J. Ch., Weiss, U., Salemink, C. A., Arnone, A., Merlini, L. and Nasini, G. (1971) *Chem. Commun.* 1463.
4. Yamazaki, S. and Ogawa, T. (1972) *Agr. Biol. Chem.* **36,** 1707; Merlini, L. and Nasini, G. (in preparation).
5. Arnone, A., Camarda, L., Merlini, L. and Nasini, G. (1975) *Gazz. Chim. Ital.* **105,** 1093.
6. Turner, W. B. (1971) *Fungal Metabolites*, p. 118. Academic Press, London.
7. Aldridge, D. C., Galt, S., Giles, D. and Turner W. B. (1971) *J. Chem. Soc.* **C,** 1623.
8. (a) Burrows, B. F., unpublished, quoted in ref. [7]; (b) Sasaki, M., Kaneko, Y., Oshita, K., Takamatsu, H., Asao, Y. and Yokotsuta, T. (1970) *Agr. Biol. Chem.* **34,** 1296; Cole, R. J., Moore, J. H., Davis, N. D., Kirksey, J. K. and Diener, U. L. (1971) *J. Agr. Food Chem.* **19,** 909.
9. Arakawa, H., Torimoto, N. and Masui, Y. (1969) *Liebigs Ann. Chem.* **728,** 152.
10. Horeau, A. and Kagan, H. B. (1964) *Tetrahedron* **20,** 2431.
11. Stothers, J. B. (1972) *Carbon-13 NMR Spectroscopy*, p. 140. Academic Press, New York.
12. Pirkle, W. H. and Dines, M. (1969) *J. Heterocycl. Chem.* **6,** 1.
13. Imagawa, T., Sueda, N. and Kamanisi, M. (1974) *Tetrahedron* **30,** 2227.
14. Kimura, Y., Katagiri, K. and Tamura, S. (1971) *Tetrahedron Letters* 3137.
15. Kikuchi, T., Mimura, T., Harimaya, K., Arimoto, T., Masada, Y., Yano, H. and Inoue, T. (1974) *Chem. Pharm. Bull.* **22,** 1946.
16. Strunz, G. M., Heissner, C. J., Kakushima, M. and Stillwell, M. A. (1974) *Can. J. Chem.* **52,** 825.