NOTES

CEPHALOCHROMIN, DIHYDROISO-USTILAGINOIDIN A, AND ISO-USTILAGINOIDIN A FROM *VERTICILLIUM* SP. K-113

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A species of *Verticillium* (strain K-113), an imperfect fungus, produced cephalochromin[1] (**Ia**), iso-ustilaginoidin A[2] (**IIa**), and a new antibiotic, dihydroiso-ustilaginoidin A (**IIIa**). The culture filtrate was adjusted to pH 3.0 with 2 N hydrochloric acid and extracted with ether. The mycelium was extracted with acetone and the acetone extract was further extracted with ether. The combined ether extracts were chromatographed on silicic acid and silica gel impregnated with 0.5 N oxalic acid to give compounds (**Ia**), (**IIa**), and (**IIIa**).

Compound (**Ia**), $C_{28}H_{22}O_{10}$ was obtained as red prisms, m.p. >300°C, $[\alpha]_D^{23}+727°$ and gave a hexa-methylether (**Ib**), yellow prisms, m.p. 227~229°C, $[\alpha]_D^{23}+310.5°$ and a hexa-acetate, yellow prisms, m.p. 178~180°C by treatment with dimethyl sulphate and with acetic anhydride in pyridine, respectively. From these data and i.r., u.v., and n.m.r. spectra, it was assumed to be cephalochromin[1].

Compound (**IIa**), $C_{28}H_{18}O_{10}$, red prisms, m.p. >300°C gave a hexa-methylether (**IIb**), m.p. 250~253°C, which was shown to be identical with an authentic sample* of ustilaginoidin A hexa-methylether[2,3] by i.r. and u.v. spectra and Rf values on t.l.c. However, its $[\alpha]_D$ value, −69°, was antipodal against that of ustilaginoidin A hexa-methylether, $[\alpha]_D+89.5°$. SHIBATA[2] converted ustilaginoidin A into its antipode, which was named iso-ustilaginoidin A, by heating under reflux in formic acid. Therefore, compound (**IIa**) was confirmed to be iso-ustilaginoidin A.

Compound (**IIIa**), $C_{28}H_{20}O_{10}$, red prisms, m.p. >300°C also gave a hexa-methylether (**IIIb**), yellow needless, m.p. 188~190°C. It is assumed by studies of u.v. and i.r. spectra of the methylether (**IIIb**) that compound (**IIIa**) is dihydroiso-ustilaginoidin A.

In order to confirm these assumption, compound (**Ib**) was dehydrogenated. When compound (**Ib**) was heated under reflux with selenium dioxide in *t*-buthanol containing one drop of pyridine, it gave two products, yellow prisms, m.p. 253~255°C and orange needles, m.p. 192~194°C. The first product was shown to be identical with ustilaginoidin A hexa-methylether by comparisons of i.r. and u.v. spectra. Its C.D. data were completely identical with those of compound (**IIb**). The first product is therefore iso-ustilaginoidin A hexa-methylether.

The second product had a molecular formula of $C_{34}H_{32}O_{10}$ and was confirmed to be compound (**IIIb**) by u.v. and i.r. spectra. Moreover, the C.D. data are identical with those of compound (**IIIb**) and the C.D. sign is in good agreement with that of iso-ustilaginoidin A hexa-methylether (**IIb**). From these results, it was shown that compounds (**Ia**) and (**IIIa**) were cephalochromin and dihydroiso-ustilaginoidin A, respectively.

Antimicrobial activity of these compounds

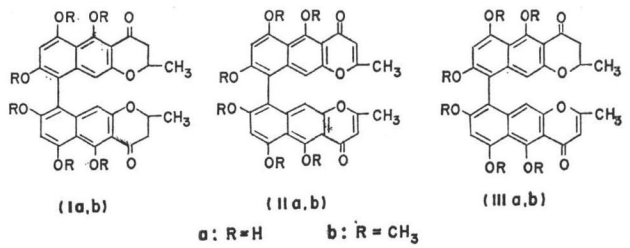

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Table 1. *In vitro* antimicrobial activity of **Ia**, **IIa**, and **IIIa**

Test organisms	MIC (mcg/ml)		
	Ia	**IIa**	**IIIa**
Bacillus subtilis PCI 219	10	10	3
Staphylococcus aureus FDA 209 P	3	10	3
Streptococcus pyogenes C-203	3	10	3

was found against Gram-positive bacteria but not against Gram-negative bacteria and fungi (Table 1).

Experimental

Isolation of Cephalochromin (**Ia**), Iso-ustilaginoidin A (**IIa**), and Dihydroiso-ustilaginoidin A (**IIIa**).

The culture filtrate (1 liter) was adjusted to pH 3.0 with 2 N hydrochloric acid and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave a crystalline residue (810 mg). The mycelium was extracted with acetone and the extract was further extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated to leave a residue (160 mg). The combined residues showed two spots, Rf 0.51 and 0.36, by t.l.c. on silica gel impregnated with 0.5 N oxalic acid (solvent system; benzene - acetone, 2 : 1). Chromatography on silicic acid (30 g) gave a cephalochromin (**Ia**) fraction (Rf 0.51, 303 mg) and a mixture (Rf 0.36) of **IIa** and **IIIa**. The mixture was rechromatographed to give an iso-ustilaginoidin A (**IIa**) fraction (4 mg), a dihydroiso-ustilaginoidin A (**IIIa**) fraction (16.5 mg), and a mixture of **IIa** and **IIIa** (86 mg).

Cephalochromin[1] (**Ia**).

The cephalochromin fraction was recrystallized from methanol to give **Ia**, red prisms (198 mg), m.p. >300°C (decomp), $[\alpha]_D^{23}$+727.0° (±40.6°)(*c* 0.189, dioxane), M^+ 518, λmax (EtOH) 235 nm (ε 43,500), 272 (47,100) 296 (54,200), 330 (15,000), 425 (11,200); νmax (KBr) 3400, 1632, 1585, 1504, 1145, 1122, 1080, 870, 840 cm^{-1}; C.D. (dioxane) $[\theta]_{210}$−49,900, $[\theta]_{225}$+115,400, $[\theta]_{265}$−458,000, $[\theta]_{294}$+500,400, $[\theta]_{326}$+56,600, $[\theta]_{340}$−50,300, $[\theta]_{415}$+11,500; n.m.r. ($CDCl_3$) δ 1.37 (CH_3, d., *J*=6 Hz), 2.65 (2H), 4.45 (1H), 5.87 (1H, s), 6.46 (1H, s) 9.54 (1H, s), 14.87 (1H, s) (Found: C, 64.55; H, 4.15; O, 30.46. $C_{28}H_{22}O_{10}$ requires C, 64.86; H, 4.28; O, 30.86 %)

Cephalochromin hexa-methylether (**Ib**) was obtained as yellow prisms (from ethyl acetate), m.p. 227~229°C, $[\alpha]_D^{23}$+310.5° (±7.6°) (*c* 0.460, dioxane), M^+ 602, λmax (EtOH) 234 nm (ε 48,900), 259 (46,500), 292 (67,700), 333 (12,200), 401 (9,600), νmax ($CHCl_3$) 1682, 1608, 1557, 1117, 1091 cm^{-1}, C.D. (dioxane) $[\theta]_{235}$+17,900, $[\theta]_{252}$−574,000, $[\theta]_{292}$+344,000, $[\theta]_{340}$−43,500, $[\theta]_{400}$+10,900 (Found: C, 67.89; H, 5.50. $C_{34}H_{34}O_{10}$ requires C, 67.76; H, 5.69 %).

Cephalochromin hexa-acetate was obtained as yellow prisms (from methanol), m.p. 178~180°C, M^+ 770 (Found: C, 62.06; H, 4.37. $C_{40}H_{34}O_{16}$ requires C, 62.33; H, 4.45 %).

Iso-ustilaginoidin A[2] (**IIa**).

The iso-ustilaginoidin A fraction was recrystallized from methanol to give **IIa**, red prisms, m.p. >300°C, M^+ 514, which was converted into the hexa-methylether (**IIb**), yellow prisms (from methanol), m.p. 250~253°C, $[\alpha]_D^{23}$−68.9° (±4.3°)(*c* 0.254, dioxane); λmax (dioxane) 229 nm (ε 40,000), 235 (33,700), 256 (64,600), 269 (59,000), 280^{sh} (74,500), 286 (87,800), 305 (4,700), 335^{sh} (7,000), 378~383 (14,000); νmax ($CHCl_3$) 1653, 1607, 1557, 1128, 1088, 1044, 952, 846 cm^{-1}; C.D. (dioxane) $[\theta]_{252}$−735,000, $[\theta]_{280}$+765,000, $[\theta]_{343}$−46,000, $[\theta]_{378}$−37,000, (Found: C, 67.91; H, 5.14. $C_{34}H_{30}O_{10}$ requires C, 68.22; H, 5.05 %).

Dihydroiso-ustilaginoidin A (**IIIa**).

The dihydroiso-ustilaginoidin A fraction was recrystallized from methanol to give **IIIa**, red prisms, m.p. >300°C, M^+ 516, which was converted into the hexa-methylether (**IIIb**), yellow needles (from methanol), m.p. 188~190°C, λmax (EtOH) 228.5 nm (ε 38,400), 257 (57,000), 286 (84,800), 315^{sh} (7,400), 327 (9,500), 341 (8,600), 381 (12,400), νmax ($CHCl_3$) 1683, 1651, 1609, 1560, 1351, 1129, 1091, 1003, 954, 859 cm^{-1}, C.D. (dioxane) $[\theta]_{253}$−543,000, $[\theta]_{285}$+647,000, $[\theta]_{342}$−55,500, $[\theta]_{375}$−14,200 (Found: C, 68.02; H, 5.16. $C_{34}H_{32}O_{10}$ requires C, 67.99; H, 5.37 %).

Dehydrogenation of Cephalochromin hexa-methylether (**Ib**) with Selenium dioxide.

A mixture of **Ib** (30 mg) and selenium

dioxide (8.3 mg) in *t*-butanol (2 ml) containing one drop of pyridine was heated under reflux for 5 hours, and then selenium dioxide (9 mg) was added to this solution. The mixture was heated under reflux for an additional 12 hours under nitrogen, and evaporated to leave a residue. The residue was separated by preparative t.l.c. on silica gel impregnated with 0.5 N oxalic acid into the starting material (**Ib**) (7.2 mg), yellow prisms, m.p. 253~255°C (4.6 mg), which was shown to be identical with iso-ustilaginoidin A hexa-methylether (**IIb**) by i.r., u.v., and C.D., and orange needles, m.p. 192~194°C (2.2 mg), which was shown to be identical with dihydroiso-utilaginoidin A hexa-methylether (**IIIb**) by i.r., u.v., and C.D.

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