METABOLIC PRODUCTS OF MICROORGANISMS. 246[†] 2880-II, A METABOLITE RELATED TO FERULIC ACID FROM *STREPTOMYCES GRISEOFLAVUS*

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By chemical screening methods we detected in the mycelium of *Streptomyces griseoflavus* (strain Tü 2880) the colabomycin-complex[2] and a second compound, called 2880-II (Rf values see Table 1). 2880-II absorbed UV light on Silica gel F_{254}, and turned black with molybdatophosphoric acid and dark brown with vanillin-sulfuric acid. It could be separated from the crude extracts by silica gel chromatography[2] and was further purified by repeated chromatography on Sephadex LH-20 (column 100×2.5 cm) in $CHCl_3$ and $CHCl_3$ - MeOH (9:1) yielding 2880-II as a yellow amorphous powder (0.2 mg/liter culture broth), which was soluble in DMF and DMSO, slightly soluble in $CHCl_3$ and insoluble in water or hexane.

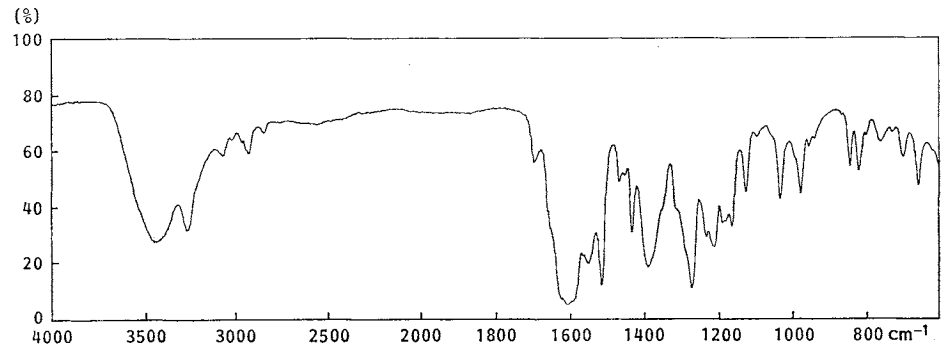
Table 1. Rf values (TLC, silica gel) of the colabomycin-complex and 2880-II.

Solvent system	Colabomycin-complex	**1**
$CHCl_3$ - MeOH (9:1)	0.46	0.39
EtOAc - MeOH - H_2O (6:2:1)	0.67	0.60

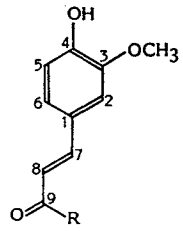
Physico-chemical properties of 2880-II are as follows: MP 272°C; IR (KBr, Fig. 1) cm^{-1} 3430, 3360, 3070, 2930, 1695, 1605 (s), 1550, 1520; UV λ_{max}^{MeOH} nm (ε) 322 (20,900), 250 (51,800); $\lambda_{max}^{MeOH-HCl}$ 338 (28,500), 268 (30,500); $\lambda_{max}^{MeOH-NaOH}$ 364 (28,800), 252 (46,100); electron impact mass spectra (EI-MS) (70 eV) *m/z* (abundance) 289 (45%, M^+, high resolution (HR) calcd for $C_{15}H_{15}NO_5$ and found: 289.0950), 177 (100%, calcd for $C_{10}H_9O_3$ and found: 177.0551), 145 (26%, calcd for $C_9H_5O_2$: 145.0289). The upfield part of the ^1H NMR spectrum (200 MHz, DMF-d_7) revealed only two methylene groups at δ 2.53 (4′-H_2 and 5′-H_2) and a methoxy singlet at δ 3.90. In the aromatic region an AX-system (J=15.5 Hz) at δ 7.28 (8-H)/7.68 (7-H) and an AMX-system (J=8 and 2 Hz) at δ 6.94 (5-H)/ 7.20 (6-H)/ 7.32 (2-H) were observed, thus indicating the presence of a trisubstituted benzene and an isolated double bond with *E*-configuration. Downfield the signals of two OH protons at δ 9.88 (4-OH)/13.78 (chelated, 3′-OH) and one NH proton (δ 9.78) occurred (all exchangeable with MeOH-d_4).

The ^{13}C NMR spectrum (50.3 MHz, DMF-d_7, attached proton test: u; up for CH_3 or CH, d; down for CH_2 or C) showed eleven resonances: δ 55.9 (u, OCH_3), 111.5 (u, C-2), 115.8 (d, C-2′), 116.1/116.3 (u/u, C-5 and C-8), 123.2 (u, C-6), 126.9 (d, C-1), 143.7 (u, C-7), 148.8 (d, C-4), 150.3 (d, C-3), 167.4 (d, C-9)[3]. With regard to

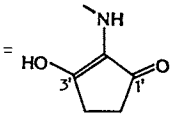
Fig. 1. IR spectrum of 2880-II (**1**) in KBr.



† See ref 1.



1 R =



2 R = OH

3 R = $NHCH_2COOH$

the molecular formula $C_{15}H_{15}NO_5$ four carbons were missing. This is typical for amide-bound 2-amino-3-hydroxycyclopent-2-enone as has been shown in the case of colabomycin A[2]. Reference to this moiety is given by the ^{13}C NMR signal for C-2′ at δ 115.8 and the ^1H NMR resonances of 3′-OH and 4′-H_2/5′-H_2.

To establish the correct substitution pattern of the trisubstituted benzene core, nuclear Overhauser enhancement (NOE) difference experiments were performed. Irradiation (DMF-d_7) at δ 3.90 (OCH_3) resulted in an intensity enhancement at 2-H (11%) only, thus indicating the neighborhood of OCH_3 to 2-H. Irradiations (DMSO-d_6) at δ 9.78 (NH) or 9.60 (4-OH) revealed intensity enhancements on 8-H (11%) or 5-H (11%), proving the spacial closeness of 8-H to NH and of 5-H to 4-OH. Due to the small difference in chemical shifts between NH and 4-OH there were less intensive NOE effects of the neighboring system, respectively. All these findings are consistent with (*E*)-*N*-(3-hydroxy-1-oxocyclopent-2-en-2-yl)-3-(4-hydroxy-3-methoxyphenyl)propenamide (=2880-II) represented by formula **1**.

From the structural point of view 2880-II could be thought to be derived biosynthetically from ferulic acid (**2**), possibly as part of soybean meal[4], and 2-amino-3-hydroxycyclopent-2-enone, generated from succinate and glycine *via* 5-aminolevulinic acid[5]. Thus the formation of 2880-II can be understood as directed biosynthesis using ingredients of soybean meal in the culture-medium. *N*-Feruloylglycine (**3**) was suspected to be a starter for the protein-biosynthesis in barley[6] and might also be a building block of soybean protein. In our case the biosynthesis of the C_5N moiety might start from this precursor and proceed by addition of succinate and subsequent cyclization using a typical pathway of secondary metabolism. On the other hand ferulic acid could be linked to the already built C_5N moiety by a nonspecific amidase, as proposed for antibiotics of the manumycin group[5] *e.g.* colabomycin[2], which is produced by the same strain. In disc-diffusion assays against Gram-positive and Gram-negative bacteria, yeasts and fungi 2880-II showed no significant inhibitory activity up to 1 mg/ml. Ferulic acid is well known for its allelopathic interference with *e.g.* soybeans[7] if exposed to the roots.

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