PREPARATION OF FLUORINATED ANTIBIOTICS FOLLOWED BY ^{19}F NMR SPECTROSCOPY

III. ACCUMULATION OF 3a-HYDROXY-6-FLUOROINDOLINE UPON ADDITION OF 6-FLUOROTRYPTOPHAN TO THE CULTURED BROTH OF *STREPTOMYCES* SP. H-63

Sir:

In the preceding papers[1,2], we reported the preparation of some fluorinated antibiotics, fluorovulgamycins and fluoroactinomycins, by the combined use of fluorinated precursors and ^{19}F NMR spectroscopy. During the course of the investigation on the preparation of fluoroactinomycin X_2, 5-fluorotryptophan proved to be incorporated into fluoroactinomycin X_2, while 6-fluorotryptophan (6FTrp) was not. This could be reasonably explained by considering the biosynthetic pathway[3,4] of actinocin with C-6 of tryptophan corresponding to C-6 of actinocin to be methylated. Therefore, the fluorine atom at C-6 in tryptophan would inhibit the formation of 6-fluoroactinomycin.

In spite of failure to produce fluoroactinomycin X_2, two fluorine resonances were observed in the ethyl acetate extract of the fermentation broth of *Streptomyces* sp. H-63 supplemented with 6FTrp. In this paper, isolation and structure determination of metabolites responsible for these signals are reported.

Streptomyces sp. H-63 was grown at 27°C in 500-ml Erlenmeyer flasks, each containing 100 ml of a medium consisting of soluble starch 2.5%, soybean meal 1.5%, dry yeast 0.2% and $CaCO_3$ 0.4%. After 48 hours of cultivation, 6FTrp was added to the culture medium at the concentration of 300 μg/ml and the cultivation was continued for a further 48 hours. Two fluorine signals (δ_F −125.0 and −114.0) derived from the metabolites of 6FTrp were recognized in the ethyl acetate extract of the fermentation broth by the use of ^{19}F NMR spectroscopy. The isolation procedure of these metabolites is depicted in Fig. 1.

The filtered broth (1 liter) was extracted with EtOAc (400 ml) and the organic layer was evaporated to dryness. The oily residue thus obtained was dissolved in MeOH and applied to a Sephadex LH-20 column which was developed with MeOH. The combined fraction showing a fluorine signal at δ_F −125.0 was concentrated. Further purification was carried out by preparative TLC using $CHCl_3$ - MeOH (85: 15) as a developer, yielding purified **1** (2 mg). Another metabolite, **2**, was purified in the same way to give a pure form (1 mg).

Physico-chemical properties of **1** were as follows: UV λ_{max}^{MeOH} nm (ε) 219 (23,000), 282 (4,300); EI-MS *m/z* 179 (M^+). The ^1H NMR spectral data (DMSO-d_6) showed the following signals: 7.48 ppm (1H, dd, J=9 and 5 Hz), 7.12 (1H, d, J=1 Hz), 7.10 (1H, dd, J=10 and 2 Hz), 6.80 (1H, dt, J=9 and 2 Hz), 4.60 (1H, t, J=5 Hz), 3.63 (2H, m), 2.80 (2H, dd, J=6 and 1 Hz). This compound proved to be identical with 6-fluorotryptophol by comparison of the ^1H NMR spectra between **1** and 6FTrp (data not shown).

Compound **2** showed absorption maxima at 242 nm (ε=7,000) and 293 (ε=2,000) in the UV spectrum. The molecular formula of **2** was established as $C_{10}H_{10}O_2NF$ by high resolution mass spectroscopy (found: 195.0706; calcd for $C_{10}H_{10}O_2NF$: 195.0696) and NMR spectra. The ^1H NMR spectrum in CD_3OD (Fig. 2) showed the following signals; 7.19 ppm (1H, dd, J=8 and 5 Hz), 6.39 (1H, m) and 6.27 (1H, dd, J=10

Fig. 1. Isolation procedures of 6-fluorotryptophol and 3a-hydroxy-6-fluoroindoline (HFI).

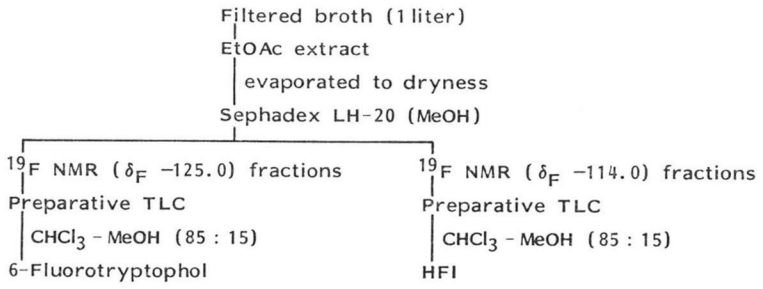


Fig. 2. The ^{1}H NMR spectrum of 3a-hydroxy-6-fluoroindoline.

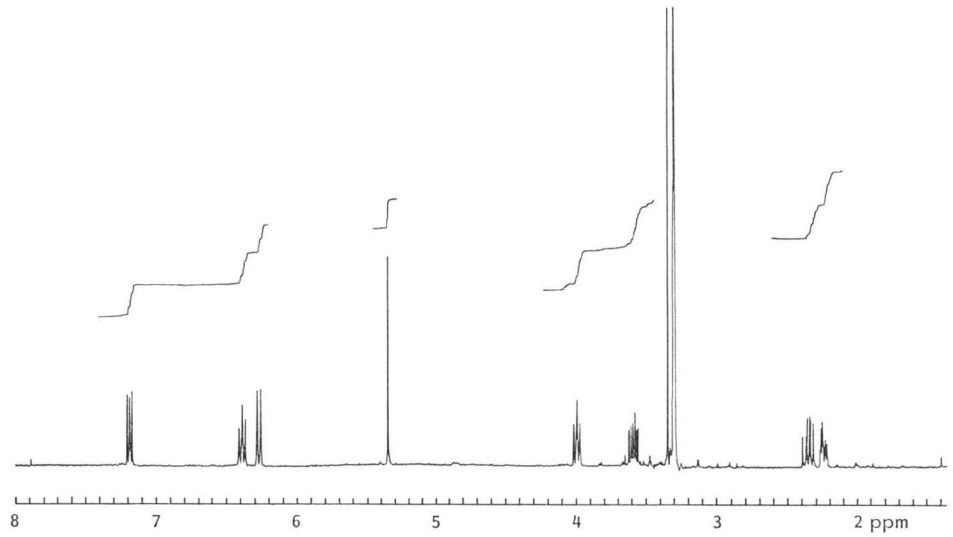
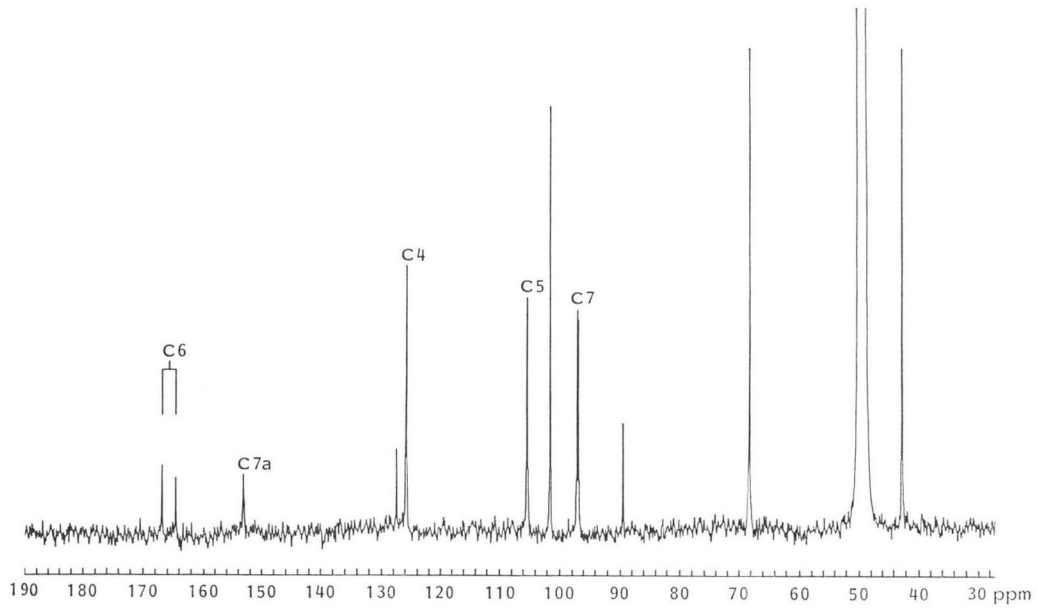
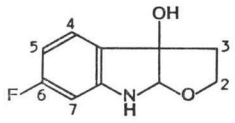


Fig. 3. The ^{13}C NMR spectrum of 3a-hydroxy-6-fluoroindoline.
The spin couplings with a fluorine atom are observed in the aromatic carbon region except for C3b.



and 2 Hz) ascribed to a 4-fluoro-1,2-disubstituted benzene judging from the ^{1}H NMR spectrum of 6FTrp; 4.04 (1H, ddd, J=9, 7 and 2 Hz), 3.64 (1H, ddd, J=10, 9 and 5 Hz), 2.04 (1H, ddd, J=12, 10 and 7 Hz) and 2.28 (1H, ddd, J=12, 5 and 2 Hz) assignable to the partial structure, -OCH_2CH_2-; 5.35 (1H, s) due to an oxymethine. The ^{13}C NMR spectrum of **2** is shown in Fig. 3. From the partial structures mentioned above, direct and long range couplings with a fluorine atom are to be observed in the ^{13}C NMR spectrum. As expected, five carbon signals are split by a fluorine into doublets which are observed at δ_C 165.6 (J_{C-F}=241 Hz), 153.0 (J_{C-F}=12 Hz), 125.8 (J_{C-F}=9 Hz), 105.3 (J_{C-F}=21 Hz) and 96.8 (J_{C-F}=26 Hz). From the accumulated

Fig. 4. Structure of 3a-hydroxy-6-fluoroindoline.



data described above, the structure of **2** was established to be 3a-hydroxy-6-fluoroindoline (HFI) as shown in Fig. 4. Although 3a-hydroxyindoline (HI) was already synthesized through a photosensitized oxygenation of tryptophol by SAITO *et al.*[5], this is the first report of the isolation of a HI derivative as a natural product. It should be emphasized that HFI was not detectable until ^{19}F NMR was employed.

HI and HFI displayed anti-platelet aggregation activities in a system[6], using adenosine diphosphate as an aggregating agent. On rabbit platelet aggregation, inhibitory activities of HFI and HI at 2 mM were 76.8% and 9.1%, respectively (papaverine hydrochloride inhibited rabbit platelet aggregation completely at 0.2 mM). It is to be noted that introduction of a fluorine atom resulted in the enhancement of anti-platelet aggregating activity of HI. No inhibition activities of HI and HFI was observed at the concentration of 100 μg/ml against *Bacillus subtilis* and *Escherichia coli*.

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