

## ISOLATION AND STRUCTURE ELUCIDATION OF A NEW COMPOUND CHETHOXYROL FROM *HILSA ILISHA*

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*Hilsa ilisha* Ham (Clupeidea) is a favorite fish of Sindhis, locally known as "Palla." It is a marine fish and ascends the rivers at the advent of flood. In Sind it begins to ascend in January, and its downward journey starts by September. It has a very high content of lipids, especially from January to June (1).

As a part of systematic biochemical investigation of *H. ilisha* collected in winter from the Indus river, we report here the isolation and structure elucidation of the new compound chethoxyrol [1] from the petroleum ether extract of the liver. The novel feature of the compound is the presence of an unusual naturally occurring acyl group at C-3 as determined through mass spectrometric and  $^1\text{H}$ -nmr studies.

Chethoxyrol has the molecular formula  $\text{C}_{31}\text{H}_{52}\text{O}_3$  (hrms). Its ir spectrum showed peaks for carbonyl stretching at  $1755\text{ cm}^{-1}$  and C-H stretching at  $2900\text{ cm}^{-1}$ . The mass spectrum showed a molecular ion at  $m/z$  472 and a base peak at  $m/z$  368 for the fragment of composition  $(\text{C}_{27}\text{H}_{44})^+$  corresponding to  $\text{M}^+ - \text{CH}_3\text{CH}_2\text{OCH}_2\text{COOH}$ . The presence of the ethoxyacetate group was further supported by  $^1\text{H}$ -nmr spectroscopy, which showed a singlet at  $\delta$  4.31, a two-proton quartet at  $\delta$  3.58, and a three-proton triplet at  $\delta$  1.24 attributed to  $\text{O}-\text{CH}_2-$

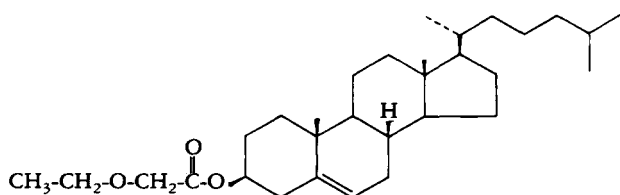
$\text{CO}$ ,  $\text{CH}_3-\text{CH}_2-\text{O}$ , and  $\text{CH}_3-\text{CH}_2-\text{O}$ , respectively. The remaining part (cholest-5-ene- $3\beta$ -oxide) of the molecule was identified by comparison with the published data for similar compounds (2-4) and by the hydrolysis of 1 into cholesterol.

### EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURE.**—The melting point was recorded in a glass capillary tube and is uncorrected. Ir ( $\text{CHCl}_3$ ) and uv (MeOH) spectra were measured on JASCO IRA-1 spectrometer and Pye-Unicam SP-800 spectrometers, respectively. Mass spectra were recorded on Finnigan MAT 112 and MAT 312 double focusing mass spectrometers connected to a PDP 11/34 computer system.  $^1\text{H}$ -nmr spectra were recorded in  $\text{CDCl}_3$  on a Bruker Aspect 300 with TMS as internal reference. The purity of sample was checked on tlc (Si gel  $\text{SiF}_{254}$  precoated aluminium plate).

**MATERIAL.**—The fish *H. ilisha* was collected from the Indus river Sind, Pakistan, in January 1986, and identified by Dr. M.M. Rabbani, National Institute of Oceanography, 37-K/6 P.E.C.H.S., Karachi-29, Pakistan, where the voucher specimen is deposited.

**ISOLATION.**—The livers (7 kg) of *H. ilisha* were homogenized and repeatedly extracted with petroleum ether. The petroleum ether solution was washed with  $\text{H}_2\text{O}$  and repeatedly shaken with 90% MeOH. The methanolic fraction was diluted with  $\text{H}_2\text{O}$  to 50% and extracted successively with petroleum ether and  $\text{Et}_2\text{O}$ . The ethereal layer was washed with  $\text{H}_2\text{O}$ , dried



( $\text{Na}_2\text{SO}_4$ ), and concentrated in vacuum. The residue thus obtained was subjected to tlc (Si gel,  $\text{CHCl}_3\text{-C}_6\text{H}_6\text{-MeOH}$ , 7:2.8:0.2), resulting in the isolation of chethoxyrol (yield, 5 mg,  $7.14 \times 10^{-3}\%$ ), as an off-white crystalline solid that, on recrystallization from  $\text{EtOAc-MeOH}$  (1:1), formed rectangular plates; mp  $82\text{-}83^\circ$ ;  $[\alpha]^{20}_D = -53.8^\circ$  ( $\text{CHCl}_3$ ); hrms  $m/z$  (rel. int. %) 472.3918 ( $\text{M}^+$ , calcd for  $\text{C}_{31}\text{H}_{52}\text{O}_3$  472.3916) (8), 385.3468 ( $\text{C}_{27}\text{H}_{45}\text{O}^+$ ) (5), 368.3451 ( $\text{C}_{27}\text{H}_{44}^+$ ) (100), 353.3204 ( $\text{C}_{26}\text{H}_{41}^+$ ) (30), 260.2501 ( $\text{C}_{19}\text{H}_{32}^+$ ) (32), 255.2119 ( $\text{C}_{19}\text{H}_{27}^+$ ) (28), 247.2430 ( $\text{C}_{18}\text{H}_{31}^+$ ) (36), 213.1638 ( $\text{C}_{16}\text{H}_{21}^+$ ) (22), 147.1170 ( $\text{C}_{11}\text{H}_{15}^+$ ) (68) and 121.1018 ( $\text{C}_9\text{H}_{13}^+$ ) (30); ir 2900 (C-H stretching) and 1755 (C=O stretching)  $\text{cm}^{-1}$ ;  $^1\text{H nmr } \delta$  5.37 (1H, d,  $J=4.2$  Hz, H-6), 4.70 (1H, dddd,  $J_{2\alpha,3\alpha}=4.2$  Hz,  $J_{2\beta,3\alpha}=9.2$  Hz,  $J_{3\alpha,4\alpha}=3.4$  Hz,  $J_{3\alpha,4\beta}=12.1$  Hz, H-3 $\alpha$ ), 4.31 (2H, s, O- $\text{CH}_2\text{-CO}$ ), 3.58 (2H, q,  $J=7.0$  Hz,  $\text{CH}_3\text{-CH}_2\text{-O}$ ), 1.24 (3H, t,  $J=7.0$  Hz, O- $\text{CH}_2\text{-CH}_3$ ), 1.02 (3H, s, H-19), 0.91 (3H, d,  $J=6.5$  Hz, H-21), 0.864 (3H, d,  $J=6.5$  Hz, H-26), 0.861 (3H, d,  $J=6.5$  Hz, H-27), 0.67 (3H, s, H-18).

**HYDROLYSIS OF 1 INTO CHOLESTEROL.**—Chethoxyrol (3 mg) was refluxed with 5% NaOH

for 15 min on a boiling water bath. The reaction mixture was then shaken with  $\text{EtOAc}$ , washed with  $\text{H}_2\text{O}$ , and dried over  $\text{Na}_2\text{SO}_4$ . On removal of the solvent, cholesterol was obtained as a white crystalline solid, mp  $149\text{-}150^\circ$ . Ms  $m/z$  386 ( $\text{M}^+$ );  $^1\text{H nmr } \delta$  5.36 (1H, d,  $J=4.2$  Hz, H-6), 3.52 (1H, dddd,  $J_{2\alpha,3\alpha}=4.2$  Hz,  $J_{2\beta,3\alpha}=9.2$  Hz,  $J_{3\alpha,4\alpha}=3.5$  Hz,  $J_{3\alpha,4\beta}=12.0$  Hz, H-3 $\alpha$ ), 1.02 (3H, s, H-19), 0.92 (3H, d,  $J=6.5$  Hz, H-21), 0.863 (3H, d,  $J=6.5$  Hz, H-26), 0.860 (3H, d,  $J=6.5$  Hz, H-27), 0.69 (3H, s, H-18).

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Received 2 June 1986