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STRUCTURE OF THE MAJOR METABOLITE OF 1-ALLYL-3,5-DIETHYL-6-CHLOROURACIL — NEW BICYCLIC BARBITURIC ACID DERIVATIVES+

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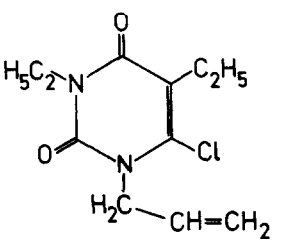
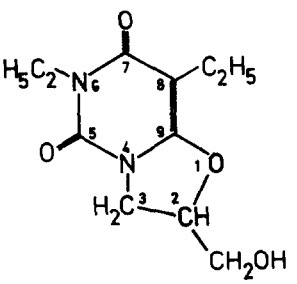
1-Allyl-3,5-diethyl-6-chlorouracil[1,2](**I**) is the active component of ACLURACIL®, a drug developed for the external treatment of herpes simplex and other viral infections of the skin and mucous membranes. Upon application to rats and rabbits, **I** is bio-transformed into two minor and a major metabolite (70-80%). The latter was isolated from rabbit urine by extraction with ether, purified by preparative thick layer and liquid column chromatography (m.p. 163-164°C, from methanol). By the combined evidence of mass and ^{1}H NMR spectroscopy, structure **II**, 6,8-diethyl-2-hydroxymethyl-tetrahydrooxazolo[3,2-c]pyrimidine-5,7(4H,6H)-dione, has been established for the major metabolite.

(**I**)





(**II**)

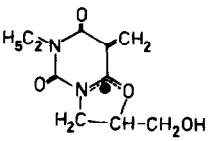
A bicyclic barbituric acid derivative of this type as a product of biochemical degradation has not yet been reported in the literature. In the metabolic transformation of **I**, the first step is epoxidation of the allylic double bond followed by hydrolysis to the propane-2,3-diol as described for analogous compounds[3]. Subsequently, the β-hydroxy group substitutes the heterocyclic chlorine,

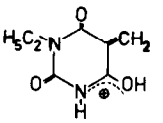
+ Dedicated to Mr. Ernst Mauz, Managing Director of ROBUGEN, on the occasion of his 75th birthday.

thus forming - via an intramolecular S_N reaction - the fused bicyclic structure **II**. This tendency towards intramolecular ring closure is very pronounced; so, upon treatment with bromine, 1-allyl-3,5-diethylbarbituric acid directly yields the bicyclic 2-bromomethyl derivative[4]. This is then transformed into the ester with silver acetate, and hydrolyzed to give **II** the structure of which thus is confirmed by an independent synthesis[5]. Because of the unusual mode of formation, however, unequivocal establishment of the skeletal structure of the whole class of compounds by spectral analysis was held indispensable.

From the mass spectrum of the metabolite, the following major fragmentation pattern appears, the molecular formula for each fragment being established by high resolution mass spectrometry. Direct correlation between the fragments listed is in all cases demonstrated by the appearance of metastable peaks m*.

molecular formula	m/e (% rel.intensity)
$[C_{11}H_{16}N_2O_4]^{\oplus\cdot}$	240 (64)
↓ - CH_3	↓ m* 211
$[C_{10}H_{13}N_2O_4]^{\oplus}$	225 (100)
↓ - C_3H_4O	↓ m* 127
$[C_7H_9N_2O_3]^{\oplus}$	169 (98)
↓ - C_3H_5NO	↓ m* 57
$[C_4H_4NO_2]^{\oplus}$	98 (40)

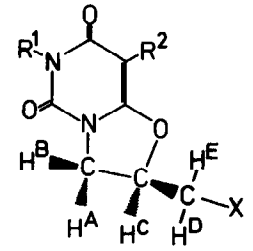




First, the β-methyl group at C-8 is cleaved off the molecular ion, leaving a highly stabilized cation. Next, the substituent at N-4 is removed: the expulsion of C_3H_4O shows one oxygen having been incorporated into the allylic side chain while the other O is bonded to the pyrimidine ring system. The loss of ethyl isocyanate in the final sequence is characteristic of uracil and barbituric acid derivatives with a N-3 ethyl group[6,7,8].

In the ^1H NMR spectrum of **II** ($CDCl_3$), a signal appears at δ 2.80 ppm (1H) which, being exchangeable with D_2O and also rather susceptible in position and line shape to both temperature and concentration, has to be attributed to an OH group. Since it is split into a triplet (J 6.0 Hz), the metabolite molecule must contain a $-CH_2OH$ moiety. The lone C-2 proton appears at lowest field, split into seven lines (not a heptuplet). The methylene protons at both C-3 and the

TABLE 1. ^{1}H NMR data of tetrahydrooxazolo[3,2-c]pyrimidine-5,7(4H,6H)-dione derivatives (δ [ppm], relative to TMS as internal standard; J [Hz]; 30°C)[a]



X	R^1	R^2	Solvent	H^C [b]	H^A	H^B	H^D,H^E	J_{AC}	J_{BC}	$J_{DC,EC}$	J_{AB}	R^1		R^2	
Br	C_2H_5	C_2H_5	$CDCl_3$ (0.1 M)	5.124	4.277	4.061	3.639[d]	8.36	6.05	5.25	-10.90	CH_2	3.962	CH_2	2.354
												CH_3	1.213	CH_3	1.072
			C_6D_6 (0.01 M)	3.794	3.265	3.207	2.470	8.44	6.17	5.29	-10.75	CH_2	4.070	CH_2	~2.51
												CH_3	1.259	CH_3	1.23
Br[c]	H	C_6H_5	$CDCl_3$ (0.003M)	5.222	4.341	4.151	3.679[d]	8.55	6.12	4.84	-10.86	N-H	~7.88	C_6H_5	multi-plet
OCH_3	C_2H_5	C_2H_5	$CDCl_3$ (0.1 M)	4.981	4.156	4.016	3.687	8.64	6.69	3.70	-10.45	CH_2	3.959	CH_2	2.356
							3.645			4.49		CH_3	1.208	CH_3	1.064
			C_6D_6 (0.1 M)	3.890	3.276	3.264	2.781	8.72	6.63	3.84	-10.30	CH_2	4.074	CH_2	2.558
							2.665			4.37		CH_3	1.247	CH_3	1.247
OH^F[e]	C_2H_5	C_2H_5	$CDCl_3$ (0.1 M)	4.982	4.173	4.100	4.030	8.59	6.94	3.35	-10.51	CH_2	3.951	CH_2	2.314
							3.831			3.71		CH_3	1.204	CH_3	1.051
			C_6D_6 (0.005M)	3.678	3.165	3.337	2.956	8.71	6.82	3.51	-10.32	CH_2	4.092	CH_2	2.540
							2.714			4.02		CH_3	1.267	CH_3	1.255

a) All spectra were measured at 90 MHz by Pulse-Fourier-Transform technique (8k interferogram, dwell time 560 or 840 μsec); number of scans varied between 1000 and 20 000, depending on concentration.

b) Values of δ and J given for H^A-H^F are obtained from theoretical spectra calculated with programs ITRCL1 and ITRCL2 (NICOLET users society) on a NICOLET BNC-12.

c) Ref.[4].

d) Spectra show some evidence of H^D,H^E-nonequivalence.

e) δ_{OH^F} 2.797 ppm, J_{DF} 5.48 Hz, J_{EF} 6.52 Hz.

exocyclic carbon are nonequivalent, however, rendering the spectrum unsusceptible to first order analysis. We have therefore calculated the theoretical spectra for **II** and for some closely related compounds which were prepared by independent synthetis, and fitted them to the experimental traces to within ± 0.01 Hz. Table 1 lists the data thus obtained which definitely confirm the heterobicyclic structure **II** for the metabolite. In each case, the less shielded of the C-3 protons is assigned to H^A by virtue of the larger coupling to H^C ($J_{cis} > J_{trans}$ for five-membered rings[9]).

Spectral analysis of all compounds listed in table 1 is additionally complicated by the N-CH_2 quartet being superimposed upon the partial spectra of H^A, H^B, and $H^{D,E}$. By changing the solvent from $CDCl_3$ to C_6D_6, however, all protons situated on the fused five-membered ring appear better shielded by 0.8-1.3 ppm while the signals of the N-6 and C-8 substituent protons are shifted slightly to lower field. Apparently, the benzene molecules orient themselves - as is known from other amide spectra[10] - farthest away from the negative end of the N-C-O dipole thus placing substituents at N-4 and C-9 directly within the diamagnetic shielding cone due to the arene ring current. If spectra accumulation is possible, the rather low solubility of these compounds in benzene ($< 5 \times 10^{-2}$ mole/l) is more than offset by the well separated partial spectra[11].

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