SYNTHESIS AND METABOLISM OF 5-ALKYLPYRIMIDINE-2-CARBOXYLIC ACIDS

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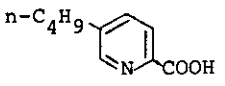
Abstract —— The metabolism of 5-propylpyrimidine-2-carboxylic acid, an azalogue of fusaric acid, has been investigated. The chain-elongated metabolites in the carboxyl group at the 2-position on the ring were identified in rat urine. The synthesis of the substrate and its main metabolite is also described.

Fusaric acid, 5-butylpyridine-2-carboxylic acid (I), isolated from a culture medium of Fusarium oxysporum,[1] is known to inhibit dopamine β-hydroxylase and to show a hypotensive effect.[2] During the investigation of the metabolism of I and related compounds, Miyazaki et al. have demonstrated[3] that 5-(3-carboxypropyl)-pyridine-2-acrylic acid (IV), 5-(3-carboxypropyl)pyridine-2-propionic acid (V), 5-carboxymethylpyridine-2-propionic acid (VI), and 5-(3-carboxypropyl)-2-pyridine-3'-oxopropionic acid (VII) are derived, together with 5-(3-carboxypropyl)pyridine-2-carboxylic acid (VIII), as metabolites of 5-(4-chlorobutyl)pyridine-2-carboxylic acid (II) in several kinds of animals.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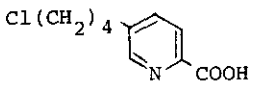
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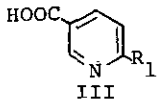
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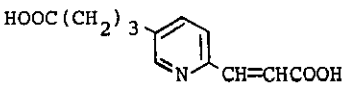
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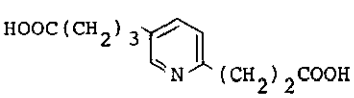
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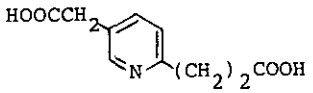
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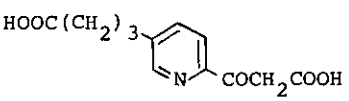
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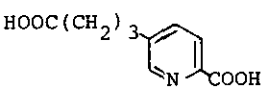
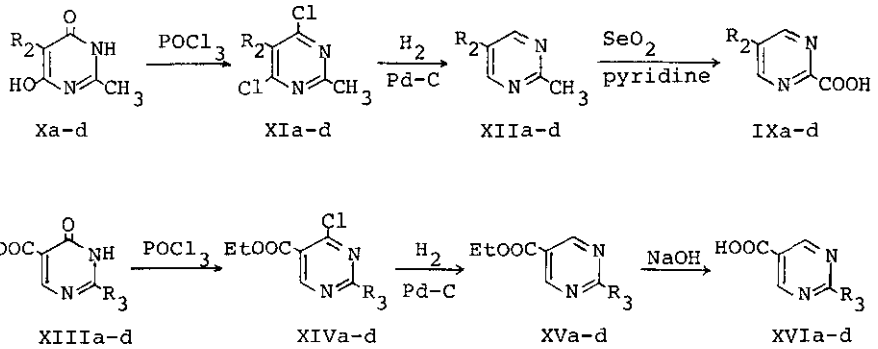

VIII

This is the first observation of the chain-elongation of compounds other than fatty acids.[4] When the metabolism of 6-alkylpyridine-3-carboxylic acids (III) and 4-alkylbenzoic acid was investigated, any chain-elongated metabolites were not detected,[3] suggesting a requirement of a nitrogen atom in the neighborhood of the carboxyl group for the elongation.

In the present paper, we wish to report the metabolism of a 3-azafusaric acid, 5-propylpyrimidine-2-carboxylic acid (IXa) and related compounds in the rat. Throughout the work, the phenomenon mentioned above was confirmed to be general in heteroaromatic compounds.



Scheme 1

The starting materials employed in the investigation were synthesized as shown in Scheme 1. 5-Alkyl-4,6-dihydroxy-2-methylpyrimidines (Xa-d) obtained by the condensation of diethyl 2-alkylmalonates with acetamidine, were treated with phosphoryl chloride in the usual manner to give the corresponding 4,6-dichloropyrimidines (XIa-d)[5]. The chlorosubstituents in XIa-d were readily removed by catalytic hydrogenation over palladium charcoal to give 5-alkyl-2-methylpyrimidines (XIIa-d). The oxidation of XIIa-d with selenium dioxide in boiling pyridine gave rise to 5-alkylpyrimidine-2-carboxylic acids (IXa-d), selectively.

Futher, diethyl ethoxymethylenemalonate was allowed to react with alkylamidines to give 2-alkyl-5-ethoxycarbonyl-4-pyrimidones (XIIIa-d)[6]. The dehydroxy-chlorination followed by the catalytic reduction transformed XIIIa-d into ethyl 2-alkylpyrimidine-5-carboxylates (XVa-d) via ethyl 2-alkyl-4-chloropyrimidine-5-carboxylates (XIVa-d). The alkaline hydrolysis of the esters (XVa-d) afforded the free carboxylic acids (XVIa-d). Physical properties and yields of the final products (IXa-d, XVIa-d) are summarized in Tables I and II.

Male Wistar rats (180-200 g) were fasted overnight and given IXa as a single oral dose of 50 mg/kg, and 0-20 hr urine specimens were collected. The urine was

Table I. Physical Properties and Yields of 5-Alkylpyrimidine-2-carboxylic Acids

No.	R_2	mp(°C)	IR $\nu_{max}^{CHCl_3}$ cm^{-1}	Yield (%) from (XI)
IXa	$n\text{-}C_3H_7$	103	1790, 1740	37
IXb	$n\text{-}C_4H_9$	129-130	1790, 1740	27
IXc	$n\text{-}C_5H_{11}$	86-87	1790, 1740	35
IXd	$n\text{-}C_6H_{13}$	89-91	1790, 1740	46

Table II. Physical Properties and Yields of 2-Alkylpyrimidine-5-carboxylic Acids

No.	R_3	mp(°C)	IR ν_{max}^{KBr} cm^{-1}	Yield (%) from (XIII)
XVIa[6]	CH_3	197-198	1730	38
XVIb	C_2H_5	182-183	1720	42
XVIc	$n\text{-}C_3H_7$	108-109	1720	37
XVId	$n\text{-}C_4H_9$	99-100	1720	39

adjusted to pH5.5 with acetic acid and chromatographed over an Amberlite CG-400 I (acetate form) column. After the column was washed with distilled water, the metabolites adsorbed on the column were eluted stepwise with acetic acid solution (0.5-4.0 M). The eluate of each fraction was evaporated to dryness under reduced pressure. The residue was treated with 5 % hydrogen chloride in methanol under reflux for 2 hrs.

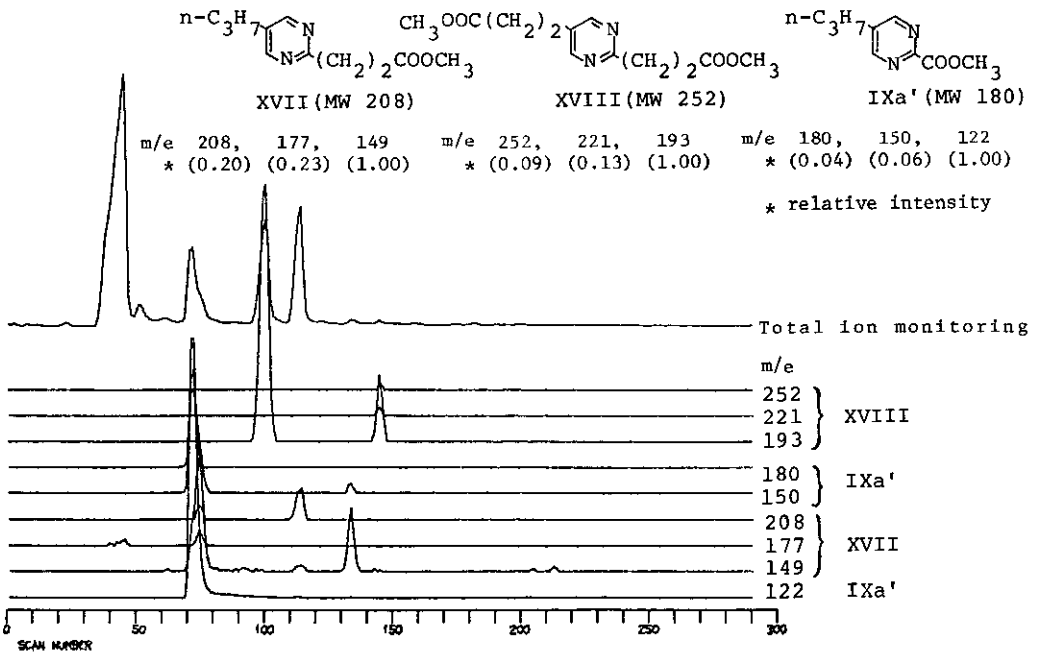
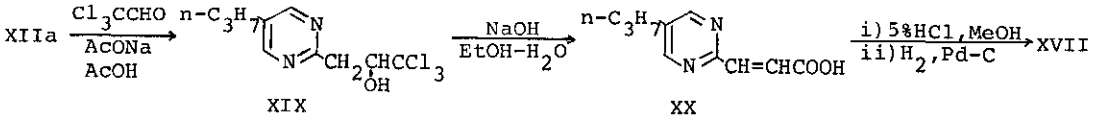


Figure Mass chromatogram of the urine extract (methyl esters).

The analysis of the resulting methyl esters of the metabolites with the aid of GC-MS exhibits the presence of methyl 5-propylpyrimidine-2-propionate (XVII) and dimethyl pyrimidine-2,5-dipropionate (XVIII), together with the methyl ester of the starting material (IXa) as shown in Figure.

In order to confirm the above observation, an authentic sample of XVII was synthesized as follows: XIIa was treated with chloral in boiling acetic acid in the presence of sodium acetate to give 5-propyl-2-(3,3,3-trichloro-2-hydroxypropyl)-pyrimidine (XIX). Alkaline hydrolysis of XIX with sodium hydroxide in aqueous ethanol afforded the corresponding acrylic acid (XX) which was converted into the methyl ester by treatment with 5 % hydrogen chloride in methanol.

Catalytic hydrogenation of the ester afforded XVII. The mass spectrum of main metabolite was consistent with that of the XVII.



Scheme 2

Since none of chain-elongated metabolites of 2-alkylpyrimidine-5-carboxylic acids (XVIa-d) were detected in the urine from rats to which these substrates were administered, it has been corroborated that the presence of a ring nitrogen atom adjacent to the carboxyl group is essential for the chain elongation reaction.

The relationship between fatty acids and heteroaromatic 2-carboxylic acids on the chain elongation is of interest. Thus, the metabolism of the N-heteroaromatic 2-carboxylic acids such as pyrazine-2-carboxylic acids, pyridazine-2-carboxylic acids and thiazole-2-carboxylic acids are investigating in our labolatory.

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