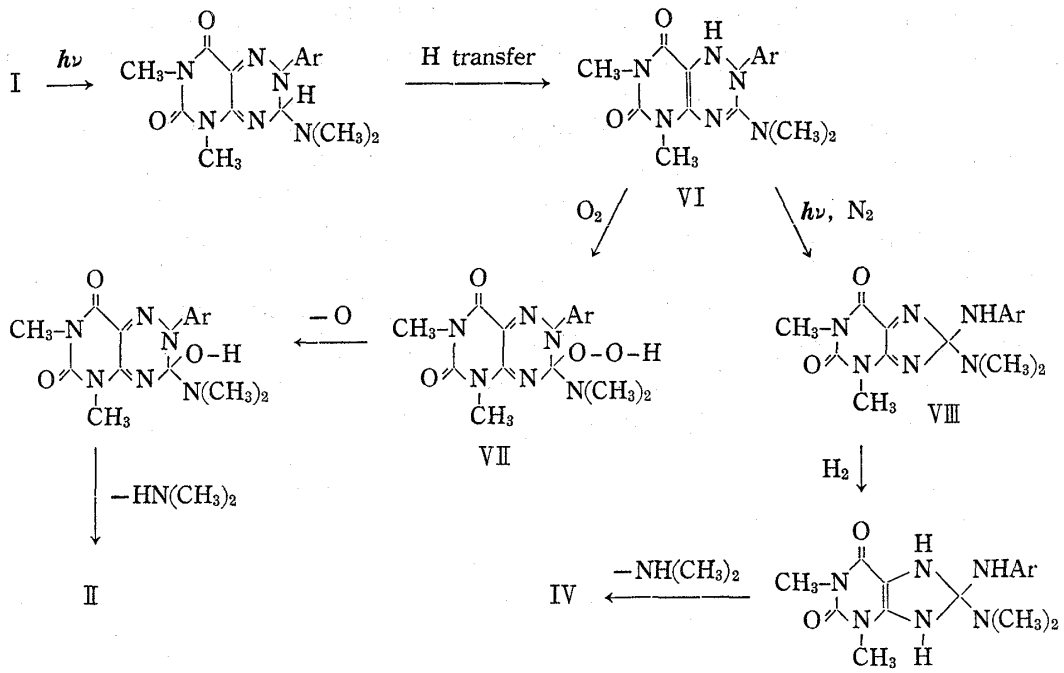

Chart 2

It is interesting to note that the thermal cyclization of Ia and Ib gave the corresponding 5-aryl-7-dimethylamino-1,3-dimethyl-5,6-dihydro-6-azalumazines (IXa and IXb) and 8-dimethylaminotheophylline (X).[1)]

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Received July 2, 1977

[Chem. Pharm. Bull. 25(10)2796—2800(1977)]

UDC 547.234.08 : 543.51.062

Quantitative Determination of Hydrazines derived from Isoniazid in Man

Hydrazine formation took place in man after the oral administration of isoniazid. The excreted amount of free hydrazine in human urine was determined quantitatively by the new method, *i.e.* mass fragmentography using ^{15}N-hydrazine as an internal standard. The unchanged isoniazid and the other metabolites, acetylisoniazid, monoacetylhydrazine and diacetylhydrazine were simultaneously analyzed.

Keywords——isoniazid–antituberculosis drug; drug metabolism; determination of free hydrazine; human urine; mass fragmentography; internal standard–^{15}N-hydrazine

Because of toxicity, mutagenicity and carcinogenicity of hydrazine, the fate of hydrazine moiety of isoniazid (INH) *in vivo* has drawn attention of some researchers since INH

was introduced as an anti-tuberculosis agent.[1-14] Although there has been a remarkable progress in the study on INH metabolism, the problem whether hydrazine formation from INH takes place in man has been left unsolved.[14] In this paper, the method of the quantitative analysis of hydrazine as well as INH, acetyl-INH, monoacetylhydrazine and diacetylhydrazine in human urine using gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) was reported.

A few of healthy adult men participated in this study as volunteers. An aqueous solution of INH (100 mg) was orally administered in the morning after 12 hr fast. The urine samples collected at 0, 2, 4, 6, 8 and 12 hr post administration were analyzed as soon as possible.

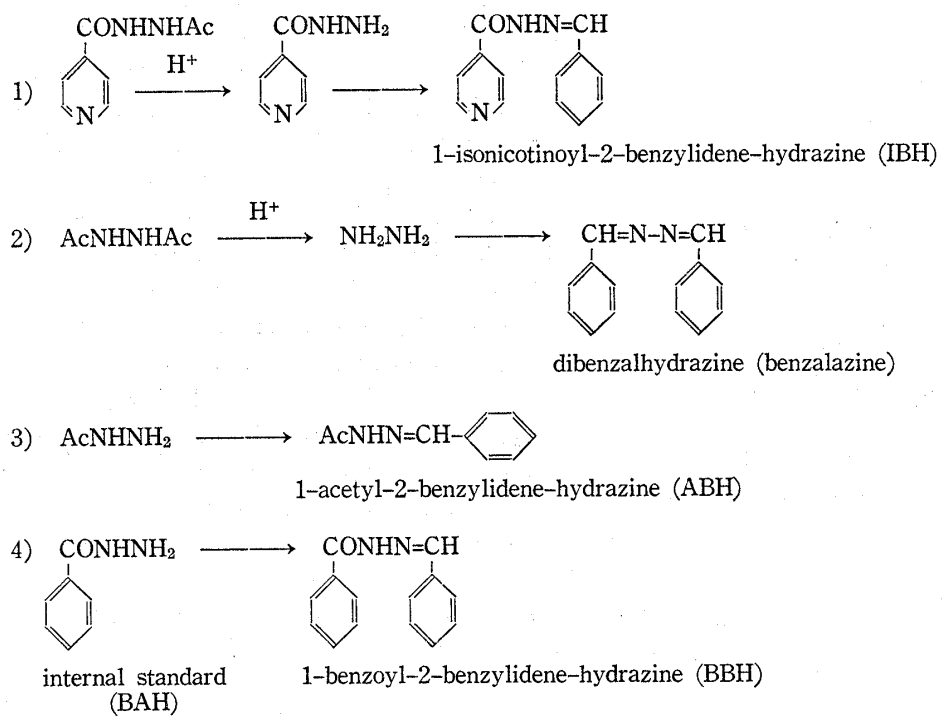

Chart 1

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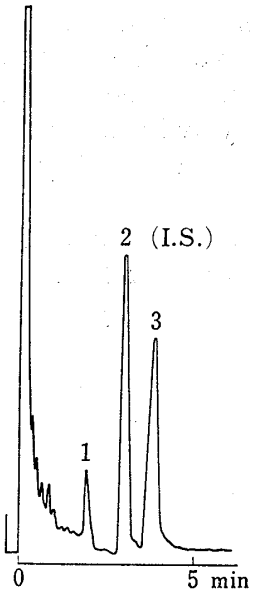

Fig. 1. Gas Chromatogram of Urine Extract after INH Administration (Subject; S.I.)

GC condition
1.5% OV-17 on Shimalite W;
3 mm × 1 m glass column;
column temp., 220°; inj.temp., 250°;
carrier gas, N_2, 20 ml/min; FID.
1: benzalazine,
Trimethylsilyl derivative of:
2: 1-benzoyl-2-benzylidene-hydrazine (BBH),
3: 1-isonicotinoyl-2-benzylidene-hydrazine (IBH).

The unchanged INH and its metabolites, acetyl-INH, monoacetylhydrazine, diacetylhydrazine and free hydrazine, were assayed as hydrazones of benzaldehyde shown in Chart 1. Free hydrazine and monoacetylhydrazine were analyzed by GC–MS because of their small amount in urine, while INH, acetyl-INH and diacetylhydrazine were determined by GC.

In order to determine unchanged INH, acetyl-INH and diacetylhydrazine, benzoic acid hydrazide (BAH) was used as an internal standard. In the case of unchanged INH, BAH and benzaldehyde were added to each of the urine sample (10 ml) adjusted to pH 4.0. After the mixture was shaken for 30 min, 1-isonicotinoyl-2-benzylidene-hydrazine (IBH) and 1-benzoyl-2-benzylidene-hydrazine (BBH) were extracted with CH_2Cl_2. The extract was dehydrated with anhydrous sodium sulfate and evaporated to dryness. The residue was used as GC sample after trimethylsilylation with N,N′-bis(trimethylsilyl) acetamide. In the case of acetyl-INH and diacetylhydrazine, 10 ml of urine was initially treated with 1 ml of conc. HCl for 24 hr at 37°. The hydrolyzed sample containing INH and free hydrazine was analyzed as IBH and dibenzalhydrazine (benzalazine) by the same method described above. The gas chromatogram of urine extract and the analytical condition were shown in Fig. 1.

Table I. Excreted Amount of INH and Its Metabolites in Human Urine after Oral Administration of INH

	Subj.	0 hr	0—2 hr	2—4 hr	4—6 hr	6—8 hr	Total (mg)	Total (mg) (INH equiv.)	% to the dose
INH[a] (mg)	H	0.000	1.920	1.000	0.000	0.000	2.920	2.920	2.9
	P_1	0.000	0.320	0.360	0.540	1.410	2.630	2.630	1.3
	P_2	0.000	1.080	9.480	17.480	18.180	46.220	46.220	23.1
Acetyl-INH (mg)	H	0.000	0.921	20.000	15.132	10.000	46.053	35.000	35.0
	P_1	##	18.553	11.316	10.000	23.421	—	—	—
	P_2	0.000	5.526	22.632	25.395	24.474	78.027	59.381	29.7
Free hydrazine (mg)	H	0.000	0.011	0.028	0.044	0.064	0.147	0.629	0.6
	P_1	##	0.051	0.033	0.026	0.143	—	—	—
	P_2	##	0.004	0.046	0.110	0.076	—	—	—
Monoacetyl-hydrazine (mg)	H	0.000	0.036	0.077	0.104	0.083	0.300	0.555	0.6
	P_1	##	0.053	0.022	0.034	0.077	—	—	—
	P_2	##	0.005	0.060	0.112	0.105	—	—	—
Diacetyl-hydrazine (mg)	H	0.000	0.280	0.424	0.547	0.182	1.433	3.381	3.4
	P_1	##	6.949	2.839	2.119	6.017	—	—	—
	P_2	##	0.318	0.886	4.610	6.127	—	—	—

a) The all values of INH include the amount of INH-hydrazones (pyruvic hydrazone, α-ketoglutaric hydrazone, *etc.*), which were hydrolyzed under our experimental condition (pH 4.0).
H: A healthy adult volunteer (male; age, 56: 100 mg of INH as an aqueous solution).
P_1: A patient on INH-treatment (male; age, 76: 400 mg of INH/day (at 9 AM and 9PM) every day for six months as powders without any other drug).
P_2: A patient on INH-treatment (female: age, 26: 400 mg of INH with 450 mg of Rifampicin and 1000 mg of Ethanbutol/day (at 9 AM and 9 PM) every day for four months as powders).
##: A remarkable detection of metabolite due to the successive administration of INH.

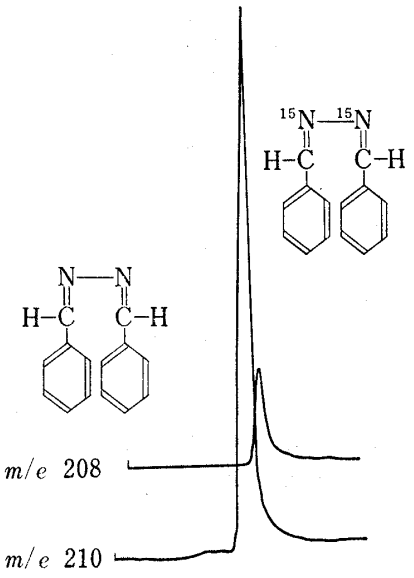

Fig. 2. Mass Fragmentogram of Dibenzalhydrazine extracted from Human Urine (Subject: S.I.)

GC condition
1.5% OV-17 on Shimalite W (80—100 mesh),
1 m × 2 mm glass column;
column temp., 185°; inj.temp., 230°.
MS condition
accelerating volt, 3 KV;
ionizing current, 300 μA;
ionizing energy, 23 eV;
separator temp., 260°.

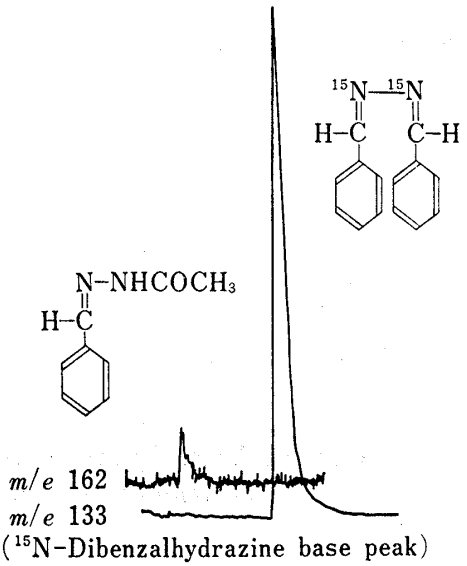

Fig. 3. Mass Fragmentogram of 1-Acetyl-2-benzylidene-hydrazine extracted from Human Urine (Subject; S.I.)

GC condition
1.5% OV-17 on Shimalite W (80—100 mesh),
1 m × 2 mm glass column;
column temp., 180°; inj.temp., 230°.
MS condition
accelerating volt, 3 KV;
ionizing current, 300 μA;
ionizing energy, 23 eV;
separator temp., 260°.

Free hydrazine and monoacetylhydrazine excreted less than a few % to the dose could be successfully determined by mass fragmentography using ^{15}N-hydrazine as an internal standard. ^{15}N-Hydrazine and benzaldehyde were added to 10 ml of urine sample (pH 4.0) to form 1-acetyl-2-benzylidene-hydrazine (ABH), benzalazine and ^{15}N-benzalazine. The solution was extracted with CH_2Cl_2. After the evaporation, the residue was used for GC–MS. In order to measure the amount of free hydrazine, the molecular ion peak height of benzalazine at *m/e* 208 was compared with that of ^{15}N-benzalazine at *m/e* 210. In the case of monoacetylhydrazine, the molecular ion peak height of ABH at *m/e* 162 was compared with the strongest peak (base ion peak) height of ^{15}N-benzalazine at *m/e* 133 (–CH=N–N=CHC_6H_5). From the peak height ratio, the amount of free hydrazine or monoacetylhydrazine was calculated. Mass fragmentogram of urine extract was shown in Fig. 2 and Fig. 3.

As the result of examination with healthy volunteers, it was ascertained that INH and its metabolites, especially free hydrazine, in urine could be determined successfully by the new method described above. Therefore, we are now examining the fate of INH in the patients on INH-treatment. The representative analytical data are summarized in Table I. Under our experimental conditions, it was concluded that INH or acetyl-INH did not liberate free hydrazine *in vitro* and that monoacetylhydrazine and diacetylhydrazine were not hydrolyzed to hydrazine. Thus, this is the first report to detect free hydrazine as one of INH metabolites in man. This observation is very valuable to answer the important question whether hydrazine formation from INH takes place in man.

The details of this study will be reported in the nearest future.

Acknowledgement The authors thank Professor Dr. H. Ibayashi and the staffs in 3rd department of Internal Medicine, Kyushu University Hospital, for urine sampling of patients on INH-treatment.

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Received July 30, 1977

[Chem. Pharm. Bull. 25(10)2800—2802(1977)]

UDC 615.277.3.011.5.015.11 : 541.69

Adaptive Least-Squares Classification applied to Structure-Activity Correlation of Antitumor Mitomycin Derivatives

An adaptive least-squares (ALS) classification which is capable of relating structure to activity rating of chemical compounds has been developed. The ALS method makes decisions for multicategory pattern classification by a single discriminant function. For the set of 16 mitomycin derivatives belonging to five activity classes used in this study, the present method is shown to be efficacious compared to other classification procedures such as the simple least-squares method, the Rao-type discriminant analysis, and the *K*-nearest neighbor method.

Keywords——structure-activity relationships; pattern recognition; nonparametric linear classifiers; mitomycin; cancer chemotherapy; drug design

The biological potency of drugs has been often represented in a form of activity rating. To such a discrete type of activity data, usual QSAR (quantitative structure-activity relationship) techniques such as the Hansch analysis[1] and the Free-Wilson approach[2] are not applicable. For the purpose of relating structure to activity class, a new classification method utilizing adaptive least-squares (ALS) technique was developed.

The ALS method makes decisions for multicategory pattern classification by a single discriminant function represented by a dot product as

$$L = w_0 + w_1x_1 + w_2x_2 + \cdots + w_mx_m = \boldsymbol{W}\cdot\boldsymbol{X} \qquad (1)$$

where $\boldsymbol{X}$ is the pattern vector $(1,x_1,x_2,\ldots,x_m)$ for m structural features of molecules, and $\boldsymbol{W}$ the weight vector $(w_0,w_1,w_2,\ldots,w_m)$. The discrimination lines between classes are fixed in advance as follows: If L_i $(=\boldsymbol{W}\cdot\boldsymbol{X}_i$; $\boldsymbol{X}_i$ is the pattern vector for the ith molecule$)\leqq 1$, then assign the ith molecule to class 1; if $1<L_i\leqq 2$, then assign to class 2; if $2<L_i\leqq 3$, then assign to class 3;....and if $L_i>k-1$, then assign to class k for classification into k classes.

The weight vector $\boldsymbol{W}$ is estimated by ALS calculation. A parameter $S^{(j)}$ described below is assumed instead of L as $S_i^{(j)}=\boldsymbol{W}\cdot\boldsymbol{X}_i$ $(i=1,2,\ldots,n)$ for the set of n compounds, and the least-squares estimate[3] of $\boldsymbol{W}$ is calculated to be $\boldsymbol{W}^{(j)}$. The superscript (j) denotes the number of iteration times. Then, $L_i^{(j)}$ $(=\boldsymbol{W}^{(j)}\cdot\boldsymbol{X}_i)$ is computed and used for classification.

$S^{(1)}$ is given by

$$S_i^{(1)} = N_i - 0.5 \qquad i = 1, 2, \cdots, n \qquad (2)$$

where N_i is the number in ascending order of activity class to which the ith molecule was observed to belong. And $S^{(j+1)}$ $(j\geqq 1)$ is adapted as

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