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Properties of Hydrazones of Hydralazine and Colorimetric Determination of Hydralazine Hydrochloride with *p*-Dimethylaminocinnamaldehyde Based on Solvent Extraction

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The hydrazones of hydralazine hydrochloride with various aromatic aldehydes were prepared and their spectral properties were examined. On the basis of the above examination, a colorimetric determination of hydralazine hydrochloride with *p*-dimethylaminocinnamaldehyde based on solvent extraction ($CHCl_3$) was developed. The calibration curve obtained was linear up to 9.8 μg/ml of hydralazine hydrochloride. The method was successfully applied for the analysis of commercial pharmaceutical preparations (tablet and injection). The contents found were 106±2% (tablet) and 100±1% (injection) of the labeled values.

Keywords——hydralazine hydrochloride; aromatic aldehyde; hydrazone; colorimetric determination; *p*-dimethylaminocinnamaldehyde; solvent extraction

Various determination methods for hydralazine (1-hydrazinophthalazine), which has been used in the treatment of essential hypertension for many years, are known.[1] The majority of the analytical methods are based on colorimetric,[2] ultraviolet (UV) absorptiometric,[3] gas-liquid chromatographic,[4] high-performance liquid chromatographic,[5] and fluorometric[6] procedures.

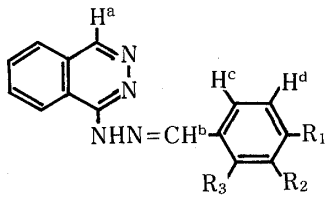
Among the colorimetric methods, the utilization of the hydrazones of hydralazine with *p*-substituted benzaldehydes (substituent: $R=NMe_2$,[2a] OH,[2b] and MeO[2c]) has already been reported. However, little systematic work has been done on the reactivity of hydralazine with various aromatic aldehydes and the properties of the resulting hydrazones.

Recently, we have reported the analysis of a series of medicaments by using color reactions of aldehydes with active methylene compounds[7] and amines.[8] In the present investigation, the preparation of the hydrazones of hydralazine with various aromatic aldehydes was first carried out, and the spectral properties of the products were determined. Among the hydrazones with a variety of *p*-substituted benzaldehydes, it was found that an interesting correlation exists between the wave numbers of the longest wavelength absorption maxima of the hydrazones and Hammett's σ_p^o-values. Next, we attempted to develop a colorimetric determination method for hydralazine hydrochloride with *p*-dimethylaminocinnamaldehyde (DMACA) based on solvent extraction ($CHCl_3$). Subsequently, the method developed was applied for the analysis of commercial pharmaceutical preparations (tablet and injection).

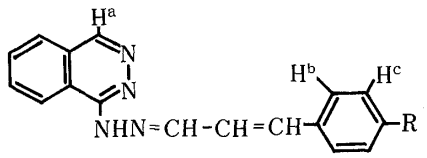
Results and Discussion

Preparation and Spectral Properties of Hydrazones (1a—i and 2a, b)

The hydrazones (Chart 1, **1a—i** and **2a, b**) were prepared by the reaction of hydralazine



1a—i



2a, b

	R_1	R_2	R_3
1a	H	H	H
1b	OH	H	H
1c	Me	H	H
1d	Cl	H	H
1e	NO_2	H	H
1f	OMe	H	H
1g	NMe_2	H	H
1h	OMe	OMe	H
1i	H	H	OH

	R
2a	H
2b	NMe_2

Chart 1

TABLE I. Hydrazones (**1a—i** and **2a, b**) of Hydralazine with Aromatic Aldehydes

Compd. No.	mp (°C)[a]	Yield (%)	Formula	Analysis (%) Calcd (Found)			
				C	H	Cl	N
1a	179—181[b]	93	$C_{15}H_{12}N_4$	72.56	4.89		22.57
				(72.55	4.85		22.40)
1b	>300 (dec.)	63	$C_{15}H_{12}N_4O$	68.17	4.58		21.20
				(68.43	4.55		21.14)
1c	195—197.5	56	$C_{16}H_{14}N_4$	73.26	5.38		21.36
				(73.51	5.38		21.31)
1d	205—209	82	$C_{15}H_{11}ClN_4$	63.72	3.92	12.54	19.82
				(63.80	3.91	12.11	19.92)
1e	235 (dec.)	80	$C_{15}H_{11}N_5O_2$	61.43	3.78		23.88
				(61.30	3.73		23.90)
1f	170—173[c]	86	$C_{16}H_{14}N_4O$	69.05	5.07		20.13
				(68.93	5.17		19.97)
1g	182—184	95	$C_{17}H_{17}N_5$	70.08	5.88		24.04
				(70.09	5.85		23.95)
1h	141—145	88	$C_{17}H_{16}N_4O_2$	66.22	5.23		18.17
				(66.36	5.21		18.15)
1i	210—212	74	$C_{15}H_{12}N_4O$	68.17	4.58		21.20
				(68.25	4.51		21.33)
2a	139—141	22	$C_{17}H_{14}N_4$	74.43	5.14		20.43
				(74.62	5.06		20.46)
2b	169—172	71	$C_{19}H_{19}N_5$	71.90	6.03		22.07
				(72.05	6.03		21.93)

a) Uncorrected. *b*) Reference 9: 177—178 °C. *c*) Reference 2*c*: 130—132 °C.

with appropriate aromatic aldehydes in EtOH (Table I). The proton nuclear magnetic resonance (^{1}H-NMR) data for the hydrazones are summarized in Table II. The absorption spectra of **1a—i** and **2a, b** were measured in several solvents and the data on the longest wavelength absorption maxima are shown in Table III; **2b** exhibited the largest molar

TABLE II. ^{1}H-NMR Spectral Data for Hydrazones (**1a**—**i** and **2a**, **b**)[a]

Compd. No.	δ (in $CDCl_3$) J=Hz
1a	7.4—8.4 (9H, m, aromatic H), 8.08 (1H, d, J=0.7, H^b), 8.45 (1H, s, H^a), 11.99 (1H, br s, NH)
1b	6.81 (2H, d, J=9, H^d), 7.83 (2H, d, J=9, H^c), 8.01 (1H, s, H^b), 8.34 (1H, s, H^a), 9.72 (1H, br s, OH), 11.78 (1H, br s, NH), 7.6—7.8 (3H, m, aromatic H), 8.2—8.3 (1H, m, aromatic H)
1c	2.35 (3H, s, Me), 7.23 (2H, d, J=8, H^d), 7.90 (2H, d, J=8, H^c), 7.7—7.8 (3H, m, aromatic H), 8.2—8.3 (1H, m, aromatic H), 8.06 (1H, d, J=0.7, H^b), 8.41 (1H, s, H^a), 11.93 (1H, br s, NH)
1d	7.47 (2H, d, J=9, H^d), 8.06 (2H, d, J=9, H^c), 8.11 (1H, s, H^b), 7.7—7.8 (3H, m, aromatic H), 8.2—8.3 (1H, m, aromatic H), 8.44 (1H, s, H^a), 12.17 (1H, br s, NH)
1e	7.7—7.8 (3H, m, aromatic H), 8.1—8.4 (6H, m, aromatic H), 8.55 (1H, s, H^a), 12.38 (1H, br s, NH)
1f	3.82 (3H, s, OMe), 7.6—7.7 (3H, m, aromatic H), 6.98 2H, d, J=9, H^d), 7.95 (2H, d, J=9, H^c), 8.03 (1H, s, H^b), 8.2—8.3 (1H, m, aromatic H), 8.38 (1H, s, H^a)
1g	2.98 (6H, s, NMe_2), 6.72 (2H, d, J=9, H^d), 7.80 (2H, d, J=9, H^c), 7.6—7.7 (3H, m, aromatic H), 7.96 (1H, s, H^b), 8.1—8.2 (1H, m, aromatic H), 8.30 (1H, s, H^a), 11.68 (1H, br s, NH)
1h	3.81 (3H, s, OMe), 3.88 (3H, s, OMe), 6.97 (1H, d, J=9, H^d), 7.35 (1H, dd, J=2 and 9, H^c), 7.82 (1H, d, J=2, R_3=H), 8.04 (1H, s, H^b), 8.2—8.3 (1H, m, aromatic H), 8.36 (1H, s, H^a), 11.98 (1H, br s, NH)
1i	6.8—7.0 (2H, m, aromatic H), 7.2—7.4 (1H, m, aromatic H), 7.7—7.8 (4H, m, aromatic H), 8.09 (1H, s, H^b), 8.3—8.4 (1H, m, aromatic H), 8.66 (1H, s, H^a), 10.26 (1H, br s, OH), 12.01 (1H, br s, NH)
2a	7.1—7.2 (2H, m, aromatic H), 7.3—7.8 (8H, m, aromatic H), 8.06 (1H, s, H^a), 8.2—8.3 (2H, m, aromatic H), 11.70 (1H, br s, NH)
2b	2.96 (6H, s, NMe_2), 6.72 (2H, d, J=9, H^c), 7.39 (2H, d, J=9, H^b), 6.8—6.9 (2H, m, aromatic H), 7.6—7.7 (2H, m, aromatic H), 7.99 (1H, s, H^a), 11.50 (1H, br s, NH)

a) Recorded on a JEOL FX 90Q instrument at 90 MHz. Chemical shifts are expressed as ppm downfield from tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, dd=double doublet, m=multiplet.

TABLE III. Absorption Spectral Data for Hydrazones (**1a**—**i** and **2a**, **b**)

Compd. No.	$\varepsilon \times 10^4$ (λ_{max} nm)			
	EtOH	$CHCl_3$	0.1 M NaOH[a]	0.1 M HCl[b]
1a	2.16 (366)	2.24 (367)	2.16 (362)	2.53 (342)
1b	2.46 (372)	2.15 (372)	3.03 (382)	2.71 (351)
1c	2.08 (368)	2.95 (371)	—[c] (363)	2.46 (347)
1d	2.32 (374)	2.30 (376)	—[c] (367)	2.85 (345)
1e	2.30 (412)	1.79 (415)	1.41 (409)	3.24 (354)
1f	2.55 (370)	2.36 (370)	2.51 (365)	2.90 (352)
1g	3.55 (391)	3.43 (396)	3.09 (387)	2.71 (340)
1h	2.75 (373)	3.01 (375)	2.72 (368)	3.00 (358)
1i	2.41 (376)	2.36 (382)	2.10 (393)	2.49 (354)
2a	3.28 (384)	2.99 (390)	3.04 (381)	4.05 (359)
2b	5.21 (414)	4.88 (419)	3.81 (403)	4.73 (356)

a) Measured in 0.1 M NaOH–5% (v/v) EtOH (9.5:1, v/v). *b*) Measured in 0.1 M HCl–5% (v/v) EtOH (9.5:1, v/v). *c*) Unstable.

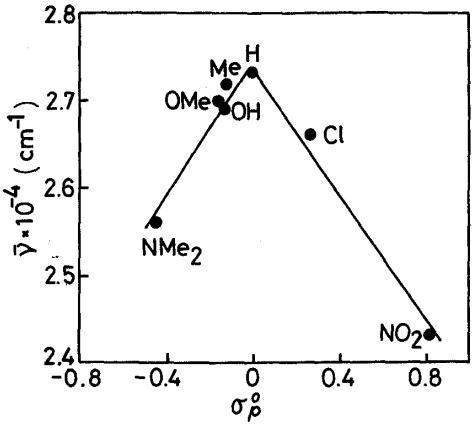


Fig. 1. Correlation between Wave Numbers of the Longest Wavelength Absorption Maxima of **1a**—**g** in EtOH and Normal Substituent Constants

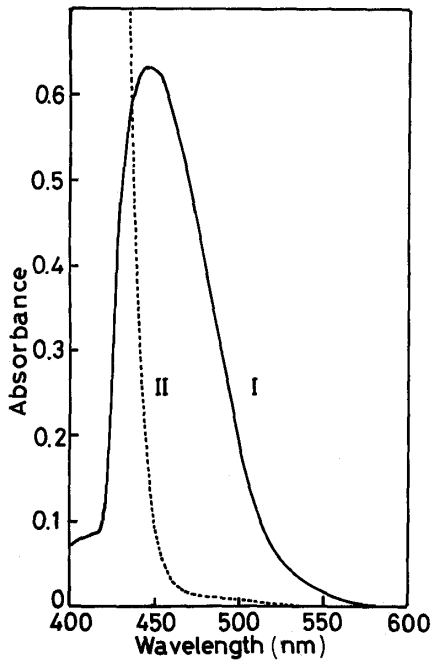


Fig. 2. Absorption Spectra

I, the hydrazone (reference: DMACA solution); II, DMACA solution (reference: $CHCl_3$).

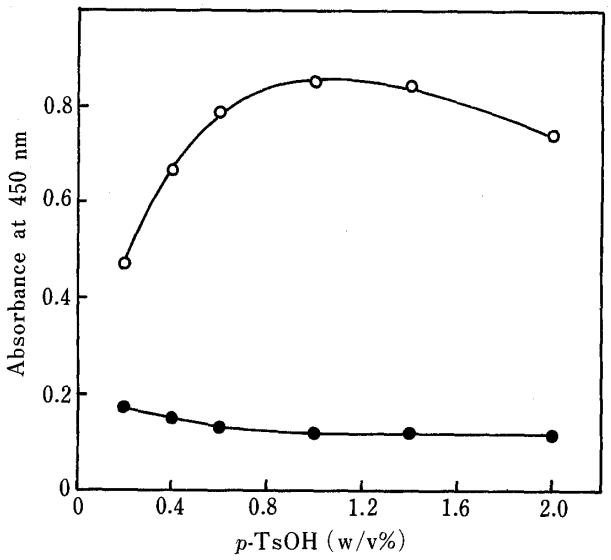


Fig. 3. Effect of TsOH

Hydralazine, 4×10^{-5} M; DMACA, 4×10^{-4} M. —○—, reaction mixture (reference: DMACA solution); —●—, DMACA solution (reference: $CHCl_3$).

absorptivity. Consequently, it was considered that DMACA could be utilized for a sensitive colorimetric determination of hydralazine as a convenient coloration reagent.

The relation between the wave numbers of the longest wavelength absorption maxima of compounds **1a**—**g** and the corresponding normal substituent constants (σ_p^o)[10] was examined. As shown in Fig. 1, it was observed that the compounds with electron-donating and electron-withdrawing groups in the *p*-position to the substituted hydrazono group behaved in different ways,[11] presumably reflecting the influence of these groups on the hydrazono moiety.

Colorimetric Determination of Hydralazine Hydrochloride with DMACA

Hydralazine condensed quite easily with DMACA in EtOH in the presence of *p*-toluenesulfonic acid (TsOH) to yield the hydrazone (**2b**), which could be extracted with $CHCl_3$ from the reaction mixture and then determined colorimetrically. The determination conditions were fully examined as described below.

The absorption spectra of **2b** extracted with accompanying TsOH against a reagent blank

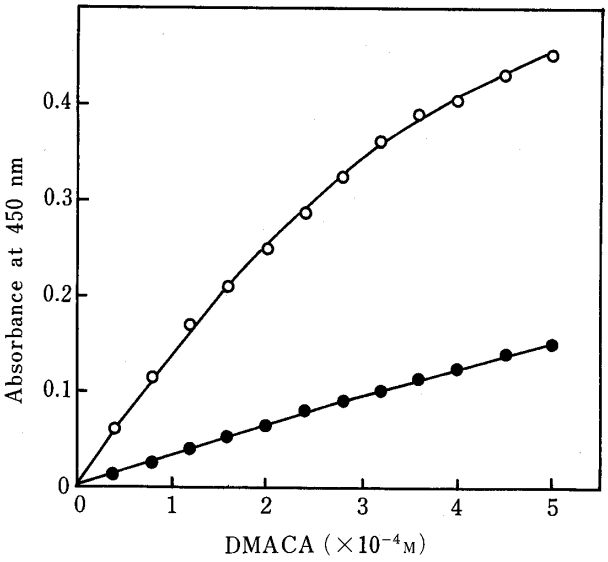


Fig. 4. Effect of DMACA Concentration on the Absorbance

Hydralazine, 2×10^{-5} M; TsOH, 1.0% (w/v). —○—, reaction mixture (reference: DMACA solution); —●—, DMACA solution (reference: $CHCl_3$).

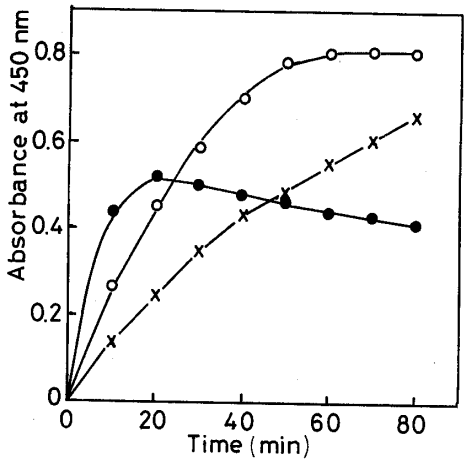


Fig. 5. Relation between Absorbance at 450 nm and Reaction Time at Several Temperatures

Hydralazine, 4×10^{-5} M; DMACA, 4×10^{-4} M; TsOH, 1.0% (w/v). —×—, at 37 °C; —○—, 60 °C; —●—, 98 °C.

prepared similarly except for the lack of test sample is shown in Fig. 2; the absorption maximum is near 450 nm. Since the isolated **2b** exhibits λ_{max} at 419 nm (Table III), this large red shift seems to be ascribable to the effect of TsOH.[12] In the subsequent study, this shift made an important contribution to reducing the influence of the background (the reagent blank).

To begin with, the effect of TsOH was examined in the range of 0.2—2.0% (w/v) TsOH for 4×10^{-5} M (1 M = 1 mol dm^{-3}) hydralazine at 450 nm (Fig. 3), and 1.0% (w/v) TsOH was selected as the optimum. Next, the effect of the reagent (DMACA) concentration was inspected in the final concentration range of $(0.4—5)\times10^{-5}$ M. As shown in Fig. 4, the absorbance of the reactants increased in proportion to the DMACA concentration, and 4×10^{-4} M DMACA (final concentration) was selected as the standard condition. The effects of temperature and time on the hydrazone formation reaction were checked by using 4×10^{-5} M hydralazine and 4×10^{-4} M DMACA (Fig. 5). About 1 h was required to obtain maximum intensity at 60 °C. For solvent extraction of **2b** from the reaction mixture, $CHCl_3$, nitrobenzene, chlorobenzene, CH_2Cl_2, CH_2ClCH_2Cl, and CCl_4 were tested. Among them, $CHCl_3$, CH_2Cl_2, and nitrobenzene proved to be effective. $CHCl_3$ was the best, and was used in this work. The color of the extract was very stable and the absorbance did not change during 24 h.

A linear relation was obtained up to 9.8 μg/ml (5×10^{-5} M) of hydralazine hydrochloride and its regression equation was expressed as follows: $y=0.094x+0.007$, y=absorbance, x= μg/ml, $c.v.=1.7\%$ ($n=5$). The correlation coefficient between absorbance value and hydralazine concentration was 0.995. The apparent molar absorptivity as estimated from the calibration curve was 2×10^4 mol^{-1} dm^3 cm^{-1} [13]

Determination of Hydralazine in Commercial Pharmaceutical Preparations

The colorimetric determination of hydralazine in commercial pharmaceutical preparations (tablet and injection) could be carried out satisfactorily according to the established standard method, as shown in Table IV. As expected from the above results, the

TABLE IV. Analytical Results for Hydralazine Hydrochloride in Commercial Pharmaceutical Preparations

No.	Tablet[a]		Injection[b]	
	Found (mg/l tablet)	Content (%)	Found (mg/l ampoule)	Content (%)
1	10.40	104.0	20.28	101.4
2	10.80	108.0	19.93	99.6
3	10.55	105.5	20.07	100.4
4	10.82	108.2	19.93	99.6
5	10.42	104.2	19.79	99.0
Mean	10.60	106.0	20.00	100.0
c.v.[c]		1.9		0.93

a) Hydralazine hydrochloride 10 mg in 1 tablet. *b*) Hydralazine hydrochloride 20 mg in 1 ampoule. *c*) Coefficient of variation.

Preparation of Sample Solutions. Tablet: A tablet was pulverized in a mortar with a pestle. About 20 mg of the powder was weighed exactly and dissolved in 100 ml of water. One ml of this solution was taken and determined according to the proposed method. Injection: One-half ml of the injection was diluted with water to prepare 20 μg/ml of hydralazine hydrochloride solution. One ml of this solution was taken and determined by the proposed method.

principal advantages of this method are that it is simple, fairly sensitive, and more convenient than other conventional methods.[2] It permits the analysis of hydralazine hydrochloride with reasonable accuracy and precision.

Experimental

Apparatus——Absorbance at a fixed wavelength was measured with a Shimadzu 150 double-beam spectrophotometer. Absorbance at various wavelengths was recorded with a Hitachi 200-10 recording spectrophotometer. Matched quartz cells of 10 mm path length were used.

Reagents and Solvents——Guaranteed reagent grade hydralazine hydrochloride (Tokyo Kasei Co., Ltd.) and *p*-dimethylaminocinnamaldehyde (DMACA) (Wako Pure Chemical Ind., Ltd.) were used. All other reagents used were of reagent grade. All solvents used were purified by distillation.

General Procedure for the Preparations of 1a—i and 2a, b——An aromatic aldehyde (0.01 mol in EtOH (50 ml)) was added to a solution of hydralazine hydrochloride (0.005 mol) in water (50 ml), and the mixture was refluxed for 20—30 min. After the solution had been cooled, the resulting precipitates (hydrazone hydrochloride) were filtered off and suspended in water (100 ml). To this suspension, a 10% (w/v) NaOH solution (0.5—1 ml) was added with stirring. The resulting hydrazone was recrystallized from MeOH or EtOH.

Colorimetric Method for Determination of Hydralazine Hydrochloride——To 1 ml of an aq. solution of the sample ($\leqq 2.5 \times 10^{-4}$ M) was added 1 ml of 5% (w/v) TsOH aq. solution, and the mixture was adjusted to the final volume (4 ml) with water. After the solution had been mixed sufficiently, 1 ml of 2×10^{-3} M DMACA in EtOH was added, and the whole was heated for 1 h at 60 °C then cooled to room temperature. The reaction mixture was extracted with $CHCl_3$ (5 ml) for 30 s with vortex mixing and the separated $CHCl_3$ layer was centrifuged for 5 min at 3000 rpm. The absorbance at 450 nm was measured against a reagent blank prepared similarly but without any sample.

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11) Rao pointed out that the absorption wavelength maxima of complementarily *p*-substituted benzene derivatives are associated with Hammett's σ (C. N. R. Rao, "Ultra-violet and Visible Spectroscopy," 2nd ed Butterworth & Co., London, 1967, Chapter 5).
12) The same phenomenon was observed on addition of TsOH to a solution of **2b** (isolated) in $CHCl_3$.
13) The decrease of the intensity in the extract, compared with the isolated hydrazone (**2b**), is perhaps related to various factors, such as the effect of TsOH, the extractability in $CHCl_3$, and the reaction yield. The reaction conditions used here were not always the optimum.