not contain an ester group or any other group that may react with the hydroxylamine reagent.

Therefore, the method is rather specific and is preferably used in the analysis of aconitine in aconite preparations that generally contain benzoylaconine and aconine. The method showed good recovery (99.8%) and a reasonable standard deviation (±0.8%) (Table II).

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Synthesis and Antituberculosis Activity of Thiocarboxamide Derivatives of Schiff Bases

VICTOR ISRAEL COHEN *[x], NOEL RIST [‡], and CHRISTIANE DUPONCHEL [‡]

Abstract □ Synthesis of some *N*-(4-thiocarboxamidobenzylidene)arylamines and *N*-(substituted benzylidene)-*p*-thiocarboxamidoanilines from various arylaldehydes and arylamines is described. Fourteen representative compounds were tested *in vitro* on the ground $H_{37}RV$.

Keyphrases □ Thiocarboxamides, various—synthesized, screened for antituberculosis activity □ Antituberculosis activity—screened in various thiocarboxamide derivatives □ Structure–activity relationships—various thiocarboxamide derivatives screened for antituberculosis activity

In a search for antituberculosis agents (1–3), some thiocarboxamide derivatives of Schiff bases of series I and II were prepared from various arylaldehydes and arylamines and studied for biological activity.

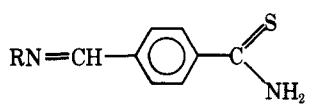
EXPERIMENTAL[1]

An attempt was made to prepare Schiff bases of series I through the thiolysis of corresponding nitriles (III) in anhydrous medium, but the reaction took an unusual path, yielding disulfide IV (4) (Scheme I). Consequently, the following route was established to synthesize the compounds: preparation of the key intermediate, *p*-thiocarboxamidobenzaldehyde, from *p*-cyanobenzylidene acetate, followed by condensation of the key intermediate with amines.

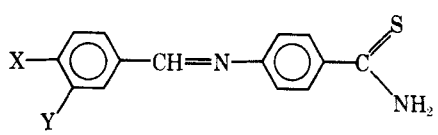
Schiff bases of series II were obtained by heating *p*-thiocarboxamidoaniline with aromatic aldehydes.

Fourteen representative compounds were tested *in vitro* on the ground $H_{37}RV$. None showed significant antituberculosis activity against either 0.01 or 0.05 mg of the bacillus at 40 μg/ml.

***p*-Cyanobenzylidene Acetate (V)**—Compound V was obtained by the method of Lieberman and Connor (5). The crude product was crystallized from alcohol and obtained in 73% yield, mp 100°.



Ia: R = C_6H_5
Ib: R = *p*-IC_6H_4
Ic: R = *p*-ClC_6H_4
Id: R = $C_6H_5CH_2$
Ie: R = *p*-$CH_3C_6H_4$
If: R = *p*-$CH_3OC_6H_4$
Ig: R = *p*-$C_2H_5OC_6H_4$
Ih: R = *p*-$CONH_2C_6H_4$



IIa: X = Y = H
IIb: X = CH_3, Y = H
IIc: X = Cl, Y = H
IId: X = CH_3O, Y = H
IIe: X = $(CH_3)_2N$, Y = H
IIf: X = H, Y = NO_2
IIg: X = S=CNH_2, Y = H

***p*-Thiocarboxamidobenzylidene Acetate (VI)**—Compound V (23.3 g, 0.1 mole) was dissolved in anhydrous pyridine (120 ml), and 20 ml of triethylamine was added. Dry hydrogen sulfide was passed through the solution for 4 hr. The reaction mixture was left at room temperature for 5 hr and was then poured into cold water and extracted with ether. The ether solution was treated with dilute hydrochloric acid, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue, which was either solid or oily, was suitable for the next step. Recrystallization from methyl ethyl ketone gave yellow crystals in 70% yield, mp 220°.

Anal.—Calc. for $C_{12}H_{13}NO_4S$: C, 53.94; H, 4.87; N, 5.24; S, 11.99. Found: C, 53.55; H, 4.90; N, 5.40; S, 12.34.

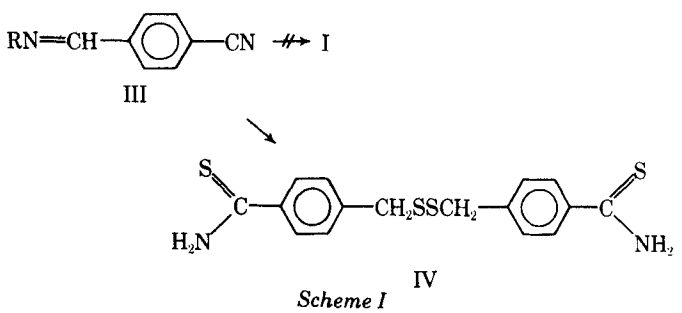
***p*-Thiocarboxamidobenzaldehyde (VII)**—Hydrolysis of VI was

[1] Melting points were measured on a Kofler hot-bench apparatus. A Beckman IR-20A spectrophotometer was used for IR spectra, which were run in potassium bromide. Microanalyses were performed by Service Central de Microanalyse, 94-Thias, France.

Table I—Physical Properties of *N*-(4-Thiocarboxamidobenzylidene)arylamines (I*a*–I*h*) and *N*-(Substituted Benzylidene)-*p*-thiocarboxamidoanilines (II*a*–II*g*)

Compound	Yield, %	Recrystallization Solvent	Melting Point	C=N, cm^{-1}	C—H, cm^{-1}	Formula	Analysis, %	Calc.	Found
I*a*	80	1-Butanol	205°	1620	3150	$C_{14}H_{12}N_2S$	C	70.00	69.86
							H	4.99	4.90
							N	11.65	11.40
							S	13.34	13.35
I*b*	75	Xylene	239°	1610	3120	$C_{14}H_{11}IN_2S$	C	45.92	46.02
							H	3.00	3.10
							N	7.64	7.53
							S	8.74	8.85
I*c*	80	Ethanol	210°	1622	3150	$C_{14}H_{11}ClN_2S$	C	61.44	61.35
							H	4.01	4.08
							N	10.23	10.28
							S	11.71	11.59
I*d*	85	Xylene	161°	1640	3150	$C_{15}H_{14}N_2S$	C	70.86	70.94
							H	5.50	5.60
							N	11.01	10.82
							S	12.61	12.81
I*e*	75	Xylene	210°	1620	3140	$C_{15}H_{14}N_2S$	C	70.86	70.98
							H	5.50	5.54
							N	11.01	10.98
							S	12.61	12.63
I*f*	80	Xylene	214°	1622	3150	$C_{15}H_{14}N_2OS$	C	66.67	66.48
							H	5.18	5.34
							N	10.36	10.08
							S	11.86	12.12
I*g*	75	Methyl phenyl ether	216°	1610	3110	$C_{16}H_{16}N_2OS$	C	67.61	67.32
							H	5.62	5.52
							N	9.85	9.66
							S	11.28	10.98
I*h*	80	Benzyl alcohol	299°	1605	3130	$C_{15}H_{13}N_3OS$	C	63.61	63.47
							H	4.59	4.66
							N	14.83	14.57
							S	11.32	11.15
II*a*	83	Ethanol	191°	1608	3110	$C_{14}H_{12}N_2S$	C	70.00	69.70
							H	4.99	5.23
							N	11.65	11.43
							S	13.34	13.30
II*b*	80	Xylene	205°	1618	3152	$C_{15}H_{14}N_2S$	C	70.86	70.94
							H	5.50	5.60
							N	11.01	11.18
							S	12.61	12.53
II*c*	75	Ethanol	217°	1624	3150	$C_{14}H_{11}ClN_2S$	C	61.44	61.16
							H	4.01	4.16
							N	10.23	10.10
							S	11.79	11.44
II*d*	85	Ethanol	202°	1623	3150	$C_{15}H_{14}N_2OS$	C	66.67	66.46
							H	5.18	5.27
							N	10.36	10.51
							S	11.86	11.92
II*e*	65	1-Butanol	276°	1615	3123	$C_{16}H_{17}N_3S$	C	67.84	67.61
							H	6.00	6.12
							N	14.52	14.57
							S	11.32	11.05
II*f*	80	Propanol	181°	1625	3185	$C_{14}H_{11}N_3O_2S$	C	58.55	58.99
							H	3.85	4.06
							N	14.72	14.62
							S	11.14	11.40
II*g*	85	Pyridine–ether	270°	1612	3130	$C_{15}H_{13}N_3S_2$	C	60.19	60.01
							H	4.34	4.35
							N	14.03	14.10
							S	21.42	21.50

accomplished according to a previous method (5). A mixture of 53.5 g (0.2 mole) of VI, 125 ml of ethanol, 125 ml of water, and 10 ml of concentrated sulfuric acid was heated to reflux for 30 min and then filtered. Water was added to the filtrate until the crystals of VII appeared. After 1 night, the solid was filtered off. The analytical sample was crystallized from xylene to yield 22.5 g (68%) of product, mp 158°.



Scheme I

Anal.—Calc. for C_8H_7NOS: C, 58.18; H, 4.24; N, 8.48; S, 19.41. Found: C, 58.35; H, 4.28; N, 8.21; S, 19.68.

***p*-Thiocarboxamidoaniline (VIII)**—Hydrogen sulfide was passed through a solution of *p*-cyanoaniline in pyridine and triethylamine (6).

***N*-(4-Thiocarboxamidobenzylidene)-*p*-iodoaniline (I*b*)**—To a solution of 2.2 g (0.01 mole) of *p*-iodoaniline in 15 ml of ethanol were added 1.65 g (0.01 mole) of VII and 3 drops of acetic acid. The mixture was refluxed for 10 min. The precipitated crystals were collected by filtration and recrystallized from xylene to give 2.8 g (76%) of pure product.

Compounds I*a*, I*c*–I*h*, and II*a*–II*g* were prepared similarly (Table I).

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TLC Determination of Iodochlorhydroxyquin and Its Conjugate in Plasma

P. DEL SOLDATO

Abstract □ A simple, specific, reliable, and sensitive method for the determination of iodochlorhydroxyquin and/or its conjugate in biological fluids is described. The method is based on a quantitative ether–acetone (1:1) extraction of plasma samples followed by TLC separation, visualization, elution, and determination at 267 nm. Iodochlorhydroxyquin released by hydrolysis of its conjugate was analyzed. Both compounds are detectable in amounts as low as 0.04 μg/ml. Application of the one-compartment open model to the data (assuming the biotransformation of the drug in conjugated form) provides a pharmacokinetic profile for the 50-mg/kg dose of iodochlorhydroxyquin in Wistar male rats.

Keyphrases □ Iodochlorhydroxyquin—and conjugate, TLC analysis, biological fluids □ TLC—analysis, iodochlorhydroxyquin and conjugate, biological fluids □ Antiamebic agents—iodochlorhydroxyquin, TLC analysis, biological fluids

Iodochlorhydroxyquin (I) has been widely used for over 30 years to treat various intestinal and vaginal infections. In view of debatable evidence of its neurotoxicity (1, 2), it is surprising that a method for the determination of I and/or its conjugate (II) in blood is not available. This paper describes a fast and simple TLC procedure for the detection of I and II in biological fluids and its use in pharmacokinetic studies.

EXPERIMENTAL

Reagents and Materials—Compound I was used as received[1]. All solvents were analytical reagent grade[2], and the developing solvent [1-butanol–acetone–diethylamine–water (30:20:4:30)] was prepared fresh daily. Silica gel plates, 250 μm thick, were activated at 105° for 1 hr[3].

Instrumentation—A 250-nm UV lamp[4], grating spectrophotometer[5], and 1-cm quartz microcells[6] were used.

Standard Preparation—Compound I was dissolved in acetone or 3 *N* HCl. Aliquots equivalent to 1, 2, 4, and 6 μg were placed in glass-stoppered centrifuge tubes and evaporated to dryness under a gentle stream of nitrogen. Plasma, 1 ml, from untreated rats was added to each tube and mixed well. These standards and the appropriate blanks were handled in the same manner as described for the plasma specimens.

Sample Preparation—One volume (1–5 ml) of plasma from treated animals and five volumes of 0.1 *N* NaOH were introduced into a 100-ml separator. Compound I was extracted with two 10-volume portions of ether–acetone (1:1 v/v).

Free I—The ether–acetone extracts were pooled, washed with five volumes of 0.1 *N* NaOH and five volumes of water, dried with sodium sulfate, and flash evaporated to about 5 ml. This final amount as well as the ether–acetone washes was transferred to a glass-stoppered centrifuge tube and evaporated to dryness under a gentle stream of nitrogen. The standards and the appropriate blanks were handled in a similar manner.

Compound II—The water phase was heated with one volume of 8 *N* HCl in a boiling water bath for 40 min, and three volumes of 3 *N* NaOH were added to the mixture. After the mixture was cooled at room temperature, I was released by hydrolysis and treated as described under *Free I*.

TLC—Each sample was taken up in 0.1 ml of acetone. Aliquots of 0.09 ml were spotted 2 cm from the edge of the chromatographic plate. Aliquots equivalent to 1.1, 2.2, 4.4, and 6.6 μg of I standard acetone solution were also spotted on the plate.

The chromatograms were developed in an ascending system of 1-butanol–acetone–diethylamine–water (30:20:4:30) in a saturated atmosphere. After 90 min, the solvent front was marked and the plates were dried at room temperature. Spots of I, visualized by exposure to UV light, were scraped into glass-stoppered centrifuge tubes, eluted with 0.6 ml of 3 *N* HCl, and shaken every 15 min for 45 min. A similar spotless area (blank) was scraped off and subjected to an identical procedure.

The test tubes were then centrifuged at 4000 rpm for 10 min, and the relative absorbance of the supernates was measured at 267 nm against a blank.

Calculations—Calibration curves were prepared by plotting absorbance at 267 nm against a known concentration of I in either plasma or 3 *N* HCl. Values for the unknown concentration of I in plasma specimens were calculated from the slope of the standard curve.

Drug Administration to Rats—Male albino Wistar rats, 200–250 g, were divided into eight groups of three animals each. Compound I was suspended in an aqueous vehicle consisting of 0.9% sodium chloride, 0.4% polysorbate 80, 0.5% carboxymethylcellulose sodium, and 0.9% benzyl alcohol and administered by gavage at a dose of 50 mg/kg in a constant volume of 5 ml/kg. Blood samples were obtained by cardiac puncture by means of a heparinized syringe at time intervals ranging from 30 min up to 24 hr after drug administration. Plasma samples were obtained by centrifugation at 2000 rpm for approximately 10 min.

Application to Pharmacokinetic Analysis—Experimental data were fitted by the method of least squares (3) applied to each exponential segment of the blood level–time curve. The extent of biotransformation of I to II was measured by employing a one-compartment open model with extravascular drug administration. The blood concentration–time curve of I after oral administration of 50 mg/kg in male rats is represented by the following biexponential equation:

$$I = I_0 \frac{K_a}{(K_a - K)} (e^{-Kt} - e^{-K_a t}) \qquad \text{(Eq. 1)}$$

[1] Gianni, Milan, Italy.
[2] Merck.
[3] Merck 60 F_{254}.
[4] Mineral Light UVS 11.
[5] Beckman DBG.
[6] Hellma 108/2 QS.