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Note

Thin-layer chromatography of sarcosine and its N-lauroyl and N-nitroso derivatives

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In recent years there has been a steady increase in the oral exposure of humans to nitrosatable secondary and tertiary amino compounds, *e.g.* during dietary intake, oral hygiene and medication, and due to habitual practices such as tobacco smoking and chewing[1,2]. Currently there is a growing awareness that dietary nitrite may interact with orally ingested amino compounds to produce carcinogenic N-nitrosamines in the oral tissues and/or in the stomach[3–9].

Sarcosine is a widely occurring secondary amino acid which readily reacts with nitrite under acidic conditions to yield N-nitrososarcosine[10–12]. The N-nitroso derivative of sarcosine is known to be carcinogenic in rats[13,14] and mice[15,16]. The sodium salt of N-lauroylsarcosine has been used as a detergent and anti-enzyme agent in dentifrices[17,18].

During our recent studies on the potential interaction between dietary nitrite and nitrosatable amino constituents of oral hygiene products[19], there was a need to develop a thin-layer chromatographic (TLC) procedure for the rapid identification of sarcosine and its N-lauroyl and N-nitroso derivatives. The present paper describes a convenient TLC procedure for monitoring the formation of N-nitrososarcosine from sarcosine and its N-lauroyl derivative under *in vitro* and *in vivo* conditions.

EXPERIMENTAL

Thin-layer chromatographic procedure

Silica gel 60 F-254 pre-coated TLC plates (E. Merck, Darmstadt, G.F.R.), layer thickness 0.25 mm, were used after activation at 105° for 5 min. Appropriate amounts of samples in water or ethanol were spotted on TLC plates and developed in solvent system A, B or C (Table I) by the ascending technique. The resolved compounds on chromatograms were detected by the following methods: (1) observing under short-wavelength ultraviolet (UV) light (254 nm), (2) exposing to iodine vapors, and (3) spraying with sulfuric acid–ethanol (1:1) solution and heating on a hot plate for 10 min.

In vitro formation of N-nitrososarcosine

Sarcosine (50 mg) and sodium N-lauroyl sarcosinate (50 mg) were incubated

separately with 100 mg of sodium nitrite in 10 ml of 10% hydrochloric acid at 37° for 4 h. The control incubations consisted of test compounds in the absence of nitrite. The reaction mixtures were adjusted to pH 11 with solid sodium hydroxide and extracted with ethyl acetate (2 × 8 ml). The ethyl acetate extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. The resulting residues were analyzed by the TLC procedure described above.

The yield of N-nitrososarcosine from the amine–nitrite reaction was determined by isolating the product; its identity was confirmed by mass spectrometry[19]. To isolate the product, preparative TLC plates (layer thickness 0.5 mm) and solvent system A were employed. The band corresponding to R_F 0.44 (visualized under the UV light) was scraped off the plate and the product isolated by extraction with 5.0% methanol in acetone. The N-nitrososarcosine was quantitated by the method of Friedman[11] based on its UV absorbance at 250 nm ($\varepsilon = 2.44 \times 10^3$).

In vivo formation of N-nitrososarcosine

Three groups of rats (Sprague-Dawley, male, weighing 100–150 g), three in each group, were administered aqueous solutions of sodium nitrite (100 mg/kg), sarcosine (100 mg/kg), and sodium nitrite together with sarcosine (100 mg/kg each), respectively, with the aid of a stomach tube. One hour after the administration, the rats were sacrificed and the stomach contents were washed into a separatory funnel with 3 ml of water. The stomach contents were then adjusted to pH 11 with solid sodium hydroxide and extracted with ethyl acetate (3 × 8 ml). The ethyl acetate extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residues thus obtained were analyzed for N-nitrososarcosine by TLC as described above. The yield of the nitroso derivative was determined by isolation of the product and its identity confirmed by mass spectrometry, as outlined under *in vitro* studies.

RESULTS AND DISCUSSION

Satisfactory TLC resolution of sarcosine and its N-lauroyl and N-nitroso derivatives was achieved in all the three solvent systems employed (Table I). Solvent system A appeared to give the best separation of the three compounds.

TABLE I

TLC OF SARCOSINE AND ITS N-LAUROYL AND N-NITROSO DERIVATIVES

Solvent systems: A, chloroform–methanol–formic acid (50:50:1); B, dioxane–chloroform–acetic acid (90:10:2); C, ethyl acetate–methanol–acetic acid (50:50:1).

Compound	R_F			*Color observed*		
	Solvent system			*Iodine*	*Sulfuric acid and heat*	*UV*
	A	*B*	*C*			
Sarcosine	0.06	0	0.05	Brown	Brown	—
N-Nitrososarcosine	0.44	0.23	0.37	Brown	Light brown	Blue
N-Lauroylsarsocine*	0.56	0.43	0.50	Brown	Dark brown	—

* Sodium salt was used.

Under the UV light, only N-nitrososarcosine was detectable as a blue spot on the chromatograms. When exposed to iodine vapors or sprayed with sulfuric acid–ethanol (1:1) solution and heated on a hot plate, all the three compounds gave brown colored spots. Thus, either of these two chromogens can be utilized for the simultaneous detection of sarcosine and its derivatives. The detection limits for all the three compounds was observed to be 20 μg.

This TLC procedure was found to be a convenient method for monitoring the *in vitro* and *in vivo* formation of N-nitrosocarcosine from the reaction of nitrite with sarcosine and its N-lauroyl derivative (Table II). Thus, development of chromatograms in solvent system A and detection of the nitroso derivative under the UV light could be used for the rapid analysis of this toxic compound in biological samples.

TABLE II

FORMATION OF N-NITROSOSARCOSINE UNDER *IN VITRO* AND *IN VIVO* CONDITIONS

Experiment	*Compounds detected by TLC*			*Yield of N-nitroso-sarcosine (%)*
	Sarcosine	*N-Lauroyl-sarcosine*	*N-Nitroso-sarcosine*	
In vitro				
Sarcosine only	+	−	−	−
Sarcosine + $NaNO_2$	+	−	+	58.7
Na N-Lauroyl sarcosinate only	+	+	−	−
Na N-Lauroyl sarcosinate + $NaNO_2$	+	+	+	6.0
In vivo				
Group I rats ($NaNO_2$ only)	−	−	−	−
Group II rats (sarcosine only)	+	−	−	−
Group III rats (sarcosine + $NaNO_2$)	+	−	+	10.5*

* Average yield from 3 rats.

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