

Trigonelline
[535-83-1]

Review of Toxicological Literature

Prepared for

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EXECUTIVE SUMMARY

The nomination of trigonelline for testing is based on its frequent occurrence in foods and the lack of carcinogenicity data.

Trigonelline is a plant hormone which is claimed to have anticarcinogenic, antimigraine, antiseptic, hypocholesterolemic, and hypoglycemic activities. It may also act as a brain sedative.

Trigonelline is widely distributed in plants within the subclass Dicotyledonae. It is also found in some species of Arthropods, Bryozoans, Cnidarians, Coelenterates, Crustaceans, Echinoderms, marine Poriferans, Molluscs, marine fishes, and mammals.

Oral preparations (prepared by hot water extraction) of fenugreek, a plant which contains trigonelline, are thought to have antipyretic and antidiarrheal properties and are claimed to strengthen nails and revitalize hair. Fenugreek is also used in the preparation of imitation maple syrup and culinary spices other than curry. Another plant containing trigonelline is tung-kua-jen, which is prepared by hot water extraction and taken orally; it is used as a diuretic and antitussive. Trigonelline is a metabolite of niacin, which is used as a hypocholesterolemic and antihyperlipidemic. For medicinal purposes, niacin is taken orally in tablet form.

Human exposure to trigonelline occurs when trigonelline-containing plants are consumed in the diet. Common foods containing trigonelline include barley, cantaloupe, corn, onions, peas, soybeans, and tomatoes. Exposure also occurs from herbal remedies, drinking coffee, and from eating fish, mussels, or crustaceans containing trigonelline. About 5% of niacin consumed is converted to trigonelline.

No production or import volumes, commercial availability data, or U.S. regulations were found for trigonelline.

Oral administration of trigonelline to female volunteers resulted in about 20% of the dose being excreted in the urine as trigonelline and about 9% of the dose being excreted as *N*-methyl-2-pyridone-5-carboxylic acid (Tg-2Py). When rats were administered trigonelline orally, all of the administered dose was recovered unchanged in the urine. No human toxicological data were found.

In rats, the oral and subcutaneous LD₅₀ doses of trigonelline are 5000 mg/kg (36 mmol/kg). Feeding 50 mg/kg (0.36 mmol/kg) trigonelline daily for 21 days to mice did not alter the weights of the liver, kidney, thymus, thyroid, adrenals, uterus, or ovaries. No visible effects were noted when cats were fed 3,500 mg (26 mmol) trigonelline for 62 to 70 days. No data were found on acute exposure, chronic exposure, or reproductive or teratological effects.

No carcinogenicity data were found, but one anticarcinogenicity study showed that trigonelline exhibited anticarcinogenic activity toward P-388 lymphocytic leukemia in mice.

Trigonelline was not mutagenic at concentrations up to 10,000 µg/plate (73 µmol/plate) in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence or absence of metabolic activation. A thermal decomposition product prepared by heating 1000 µmol trigonelline at 250°C was mutagenic in *S. typhimurium* strain TA98 in the presence of metabolic activation. Trigonelline (1000 µmol) heated in combination with individual amino acids or glucose was also mutagenic in the presence of metabolic activation. When 1000 µmol trigonelline was heated in combination with multiple amino acids and glucose, the reaction products were mutagenic in *S. typhimurium* strains TA98 and YG1024 but not strain YG1029 in the presence of metabolic activation. In the absence of S9, the heated trigonelline, amino acids, and glucose reaction products were mutagenic

in strains TA98 and YG1029 but were toxic to YG0124. Trigonelline was not mutagenic at concentrations up to 7,400 µg/plate (54 µmol/plate) in the L5178Y TK^{+/+} mouse lymphoma mutation assay, with and without metabolic activation.

No data on immunotoxicity were found.

In one experiment with rats, trigonelline reduced total and free cholesterol levels. High concentrations of trigonelline inhibited glycine betaine accumulation in osmotically stressed canine kidney cells *in vitro*.

Trigonelline counteracted the hyperglycemic effect of cortisone when administered concomitantly, but not when administered 2 hours after cortisone administration, to non-diabetic rabbits. A mild and transient hypoglycemic effect was also observed in rats with alloxan-induced diabetes treated with 250 or 1000 mg/kg (1.8 or 7.3 mmol/kg) trigonelline.

Trigonelline is involved in G2 cell cycle arrest in meristematic roots and shoots. It may function as a signal transmitter in the response to oxidative stress in plants, based on an observed increase in nicotinamide and trigonelline concentrations in plant tissues induced with UV-B or oxidative stress *in vitro*.

In terms of structure-activity, the degree of inhibition of *N*'-methylnicotinamide uptake varies among *N*'-methylnicotinamide analogs. Unsubstituted *N*-methylpyridine produced considerable inhibition, as did 1-methyl-2-acetylpyridine, 1-methyl-4-acetylpyridine, and methyl *N*-methylpyridine-3-carboxylate. The methyl, ethyl, and butyl esters of trigonelline all produced strong inhibition. Trigonelline was not inhibitory.

Niacin analogs that may be converted to niacin or nicotinamide are active alleviating agents for dicotophos-induced teratogenesis, while analogs such as trigonelline which are not likely to be converted to niacin or nicotinamide are inactive as such agents.

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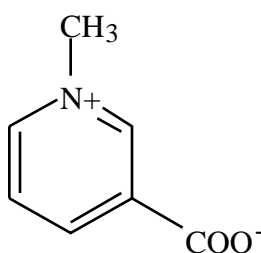
1.0 BASIS FOR NOMINATION

The nomination of trigonelline for testing is based on its frequent occurrence in high concentrations in foods and the lack of carcinogenicity data.

2.0 INTRODUCTION

Trigonelline

[535-83-1]



2.1 Chemical Identification

Trigonelline (C₇H₇NO₂, mol. wt. = 137.14) is also called:

Pyridinium, 3-carboxy-1-methyl-, inner salt (9CI)
Pyridinium, 3-carboxy-1-methyl-, hydroxide, inner salt (8CI)
Betain nicotinate
Betaine nicotinate
Caffearine
3-Carboxy-1-methylpyridinium betaine
3-Carboxy-1-methylpyridinium hydroxide inner salt
3-Carboxy-1-methylpyridinium inner salt
Coffearin
Coffearine
Gynesine
Gynesis
N-Methylnicotinate
N-Methylnicotinic acid
N'-Methylnicotinic acid
N-Methylnicotinic acid betaine
Nicotinic acid, *N*-methyl
Nicotinic acid *N*-methylbetaine
Trigenolline

Trigonellin

2.2 Physical-Chemical Properties

Property	Information	Reference
Soluble in:	Water	HODOC (1997)
Melting Point (°C)	218	HODOC (1997)

Trigonelline is a strongly polar hydrophilic compound (Gill et al., 1970; cited by Viani and Horman, 1974). Trigonelline comprises about 0.7% (*Coffea robusta*) and 1.1% (*C. arabica*) of the chemical composition of green coffee (Wasserman et al., 1993). About 50-80% of the trigonelline is decomposed during roasting, forming niacin and some aromatic nitrogen compounds, including pyridines, pyrroles, and bicyclic compounds.

2.3 Commercial Availability

No data were found.

3.0 PRODUCTION PROCESSES AND ANALYSES

Trigonelline is produced from heterotrophic cell suspension cultures of *Glycine max* (soybean) by nicotinic acid-*N*-methyl-transferase-mediated conversion of endogenous or exogenously applied niacin (Höhl et al., 1988).

4.0 PRODUCTION AND IMPORT VOLUMES

No data were found.

5.0 USES

Trigonelline, a plant hormone (Evans et al., 1979; Evans and Tramontano, 1981; both cited by Tramontano et al., 1986), is claimed to have the following therapeutic properties: anticarcinogenic (cervix and liver), antimigraine, antiseptic, hypocholesterolemic, and hypoglycemic activities (Beckstrom-Sternberg and Duke, 1997). When administered to rats, trigonelline elevated the seizure threshold, indicating that the substance may act as a brain sedative (Czok, 1974).

Fenugreek (*Trigonella foenum-graecum*), a plant which contains trigonelline, is used as an antipyretic and antidiarrheal (Der Marderosian, 1977). It is prepared by hot water extraction and is taken orally. Fenugreek is also used in making imitation maple syrup and for culinary spices other than curry; it is claimed to have emollient properties (Budavari, 1996). An oral preparation containing fenugreek extracts is claimed to strengthen nails and revitalize hair (Mai and Mai, 1997).

Other plants containing trigonelline include Tung-kua-jen (*Benincasa hispida*), which is used as a diuretic and antitussive (Li, 1974), and *Strophanthus kombe* and *S. hispidus*, which are used as poisons on arrows by African natives because of the cardiotoxic properties of the two plants (Budavari, 1996). Tung-kua-jen is prepared by hot water extraction and is taken orally (Li, 1974).

Trigonelline is a metabolite of niacin in humans (Yuyama and Suzuki, 1991), which is a component of over-the-counter vitamin supplements and is used as a hypocholesterolemic and as an antihyperlipidemic (Physicians' Desk Reference, 1995). Niacin is produced in tablet form and the typical dosage is 1 to 2 g two or three times a day.

Based on *in vivo* animal studies, trigonelline is proposed for use in humans as an effective carrier for drug delivery to the brain [e.g., phenylethylamine (Bodor and Farag, 1983), dopamine (Anonymous, 1983), 2',3'-dideoxynucleosides (Palomino et al., 1989), phenytoin (Pop et al., 1989)] and skin [e.g., acyclovir (Chikhale and Bodor, 1991)].

6.0 ENVIRONMENTAL OCCURRENCE AND PERSISTENCE

Trigonelline is widely distributed in plants within the subclass Dicotyledonae, having been identified in the dry seeds of some plants within following superorders: Araliiflorae, Caryophylliflorae, Corniflorae, Fabiflorae, Gentianiflorae, Lamiiflorae, Loasiflorae, Magnoliflorae, Malviflorae, Myrtiflorae, Polygoniflorae, Primuliflorae, Ranunculiflorae, Rosiflorae, Rutiflorae, Santaliflorae, Solaniflorae, and Violiflorae (Tramontano et al., 1986). A list of some plants containing trigonelline to which humans are exposed are listed in **Table 1** with the respective concentrations found in each.

Trigonelline is also found in jellyfish (*Verella spirans*), sea urchins (*Arabacia pustulosa*), (Budavari, 1996), the muscle of Crustacea (Leonard and Macdonald, 1963; cited by Viani and Horman, 1974), and in the marine sponges *Calyx nicaensis* (Ackermann and Pant, 1961; cited by Anthoni et al., 1991) and *Stryphnus ponderosus* (unpublished results, cited by Anthoni et al., 1991). It has been identified in Arthropoda (Dudel et al., 1963; Leonard and MacDonald, 1963; both cited by Anthoni et al., 1991), Bryozoa (Fukushima, 1962; cited by Anthoni, 1991), Chordata (Ackermann and List, 1957; cited by Anthoni et al., 1991), all three classes of Cnidaria (Anthozoe, Hydrozoa, and Scyphozoa) (Ackermann, 1953; Welsh and Prock, 1958; Gupta et al., 1977; Berking 1986; all cited by Anthoni et al., 1991), and Mollusca (Hilta, 1970; Hayashi and Konosu, 1977; Yasumoto et al., 1978; all cited by Anthoni et al., 1991). Trigonelline has been found in various organs of filefish, gizzard shad, horse mackerel, Japanese anchovy, Pacific herring, Pacific saury, round herring, and true sardines (Ito et al., 1994), and it has also been found in mammals (Ackerman, 1912; cited by Anthoni et al., 1991).

Trigonelline might occur as a minor component in wastes from industrial coffee roasting and extraction processes.

Table 1. Plants Containing Trigonelline

Common Name	Scientific Name	Plant Part	Concentration (ppm)	Reference
Barley	<i>Hordeum vulgare</i>	Seed	9	Beckstrom-Sternberg & Duke (1997)
Cantaloupe	<i>Cucumis melo</i>	Seed	2-6	
Coffee	<i>Coffea arabica</i>	Seed	3,000-13,000	Stennert and Maier (1994)
		Bean	6,000-13,000	
	<i>C. canephora</i> var. <i>robusta</i>	3,000-11,000		
	<i>C. liberica</i>		2,400-2,900	
Corn	<i>Zea mays</i>	Seed	4	Beckstrom-Sternberg & Duke (1997)
Fenugreek	<i>Trigonella foenum-graecum</i>	Seed	1,300	
Hemp (marijuana)	<i>Cannabis sativa</i>	n.p.	n.p.	Budavari (1996)
Onion	<i>Allium cepa</i>	Seed	13	Beckstrom-Sternberg & Duke (1997)
Pea	<i>Pisum sativum</i>	Seed	128-227	
		Fruit	6-203	
		Sprout Seedling	91	
		Leaf	9-88	
		Root	1-75	
		Shoot	55	
		Stem	1-24	
Soybean	<i>Glycine max</i>	Seed	19.7-71.8	
		Leaf	10.5-63.2	
		Fruit	3.7-16.2	
		Stem	1.5-7.6	
		Root	1.1	
Tomato	<i>Lycopersicon esculentum</i>	Root	69	
Tung-kua-jen	<i>Benincasa hispida</i>	n.p.	n.p.	Li (1974)

Abbreviations: n.p. = not provided

7.0 HUMAN EXPOSURE

Humans are exposed to trigonelline when trigonelline-containing plants are consumed in the diet. Common foods containing trigonelline include barley, cantaloupe, corn, onions, peas, soybeans, and tomatoes (Beckstrom-Sternberg and Duke, 1997). Trigonelline may be present in prepared coffee in concentrations as high as 1% (Taguchi et al., 1985, 1986). The average

trigonelline content in a cup of coffee is 53 mg (0.39 mmol) (Clinton, 1985; cited by IARC, 1991). Trigonelline exposure also occurs when crustaceans, fish, or mussels containing trigonelline (Ito et al., 1994) are consumed.

Trigonelline is a metabolite of niacin (a vitamin and prescribed drug), with about 5% of the niacin consumed being converted to trigonelline (Yuyama and Suzuki, 1991). Thus, if an individual consumes the recommended daily allowance of niacin (15 mg), about 0.75 mg of trigonelline is produced. According to one study, trigonelline is not a metabolite of niacytin (the bound form of niacin found in cereals) (Carter and Carpenter, 1981).

The plants fenugreek (Der Marderosian, 1977; Mai and Mai, 1997) and tung-kua-jen (Li, 1974) contain trigonelline and are taken orally as herbal remedies. Foods containing fenugreek as a spice ingredient (Budavari, 1996) are another source of exposure.

8.0 REGULATORY STATUS

Trigonelline is not included in the Toxic Substances Control Act inventory (Chemlist, 1997), and no U.S. regulations were found.

9.0 TOXICOLOGICAL DATA

Summary: Oral administration of trigonelline to female volunteers resulted in 20-21% of the dose being excreted in the urine as trigonelline and 9-10% of the dose being excreted as *N*'-methyl-2-pyridone-5-carboxylic acid (Tg-2Py). When rats were administered trigonelline orally, all of the administered dose was recovered unchanged in the urine. No human toxicological data were found.

In rats, the oral and subcutaneous LD₅₀ doses of trigonelline are 5000 mg/kg (36 mmol/kg). Feeding 50 mg/kg (0.36 mmol/kg) trigonelline daily for 21 days to Sabra albino mice did not alter the weights of the liver, kidney, thymus, thyroid, adrenals, uterus, or ovaries. No visible effects were noted when cats were fed 3,500 mg (26 mmol) trigonelline for 62 to 70 days. No data were found on acute exposure, chronic exposure, or reproductive or teratological effects.

No carcinogenicity data were found, but one anticarcinogenicity study found that trigonelline exhibited anticarcinogenic activity toward P-388 lymphocytic leukemia in mice.

Trigonelline was not mutagenic at concentrations up to 10,000 µg/plate (73 µmol/plate) in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence or absence of metabolic activation. A thermal decomposition product prepared by heating 1000 µmol trigonelline was mutagenic in *S. typhimurium* strain TA98 in the presence of metabolic

activation. Trigonelline (1000 μmol) heated in combination with alanine, arginine, cysteine, lysine, phenylalanine, proline, serine, threonine, valine, or glucose was also mutagenic in the presence of metabolic activation. When 1000 μmol trigonelline was heated in combination with a mix of amino acids and glucose, the reaction products were mutagenic in *S. typhimurium* strains TA98 and YG1024 but not strain YG1029 in the presence of metabolic activation. In the absence of S9, the heated trigonelline, amino acids, and glucose reaction products were mutagenic in strains TA98 and YG1029 but were toxic to YG0124. Trigonelline was not mutagenic at concentrations up to 7,400 $\mu\text{g}/\text{plate}$ (54 $\mu\text{mol}/\text{plate}$) in the L5178Y TK^{+/−} mouse lymphoma mutation assay, with and without metabolic activation.

No data on immunotoxicity were found.

In one experiment with rats, trigonelline reduced total and free cholesterol levels. High concentrations of trigonelline inhibited glycine betaine accumulation in osmotically stressed canine kidney cells *in vitro*.

Trigonelline counteracted the hyperglycemic effect of cortisone when administered concomitantly, but not when administered 2 hours after cortisone administration, to non-diabetic rabbits. A mild and transient hypoglycemic effect was also observed in rats with alloxan-induced diabetes treated with 250 or 1000 mg/kg (1.8 or 7.3 mmol/kg) trigonelline.

Trigonelline is involved in G2 cell cycle arrest in meristematic roots and shoots. It may function as a signal transmitter in the response to oxidative stress in plants, based on an observed increase in nicotinamide and trigonelline concentrations in plant tissues induced with UV-B or oxidative stress *in vitro*.

In terms of structure-activity, the degree of inhibition of *N*'-methylnicotinamide uptake varies among *N*'-methylnicotinamide analogs. Unsubstituted *N*-methylpyridine produced considerable inhibition, as did 1-methyl-2-acetylpyridine, 1-methyl-4-acetylpyridine, and methyl *N*-methylpyridine-3-carboxylate. The methyl, ethyl, and butyl esters of trigonelline all produced strong inhibition. Trigonelline was not inhibitory.

Niacin analogs that may be converted to niacin or nicotinamide are active alleviating agents for dicrotophos-induced teratogenesis, while analogs, such as trigonelline, that are not likely to be converted to niacin or nicotinamide are inactive as such agents.

9.1 General Toxicology

9.1.1 Human Data

No data were found.

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

When female volunteers were administered 50 mg (0.36 mmol) trigonelline orally, 20-21% of the dose was excreted in the urine as trigonelline and 9-10% was excreted as *N*'-methyl-2-pyridone-5-carboxylic acid (Tg-2Py) (Yuyama and Suzuki, 1991; Yuyama and Kawano, 1996).

In weanling rats (strain not provided) administered a large amount (dose not provided) of trigonelline orally, all of the trigonelline dose was excreted unchanged in the urine (Shibata and Taguchi, 1987). Similarly, rats (strain not provided) receiving 4,400 mg/day of whole corn excreted approximately 0.09 mg (0.00066 mmol) of trigonelline in the urine; it was concluded that the amount of trigonelline ingested from the corn was recovered almost completely in the urine (Carter and Carpenter, 1981).

9.1.3 Acute Exposure

Acute toxicity values for trigonelline are presented in **Table 2**.

Table 2. LD₅₀ Values for Trigonelline

Route	Species (sex and strain)	LD ₅₀	Reference
oral	Sabra albino rat, female	5000 mg/kg (36 mmol/kg)	Shani (Mishkinsky) et al. (1974)
s.c.	rat (sex and strain n.p.)	5000 mg/kg (36 mmol/kg)	Brazda and Coulson (1946; Proc. Soc. Exp. Biol. Med. 62:19; cited by RTECS, 1997)

Abbreviations: n.p. = not provided; s.c. = subcutaneous

9.1.4 Short-Term and Subchronic Exposure

These studies are presented in **Table 3**.

When female Sabra albino mice were fed 50 mg/kg (0.35 mmol/kg) trigonelline daily for 21 days, no changes in the weight of the liver, kidney, thymus, thyroid, adrenals, uterus, or ovaries were identified (Shani (Mishkinsky) et al., 1974). None of the test animals died during the experiment.

No visible effects were noted when cats were fed 3,500 mg (26 mmol) trigonelline daily for 62 to 70 days (Faulkner and Smith, 1950).

9.1.5 Chronic Exposure

No data were found.

9.2 Reproductive and Teratological Effects

No data were found.

9.3 Carcinogenicity

No carcinogenicity data were found. However, one anticarcinogenicity study was found, and is summarized in **Section 9.4**.

9.4 Anticarcinogenicity

This study is presented in **Table 4**.

Trigonelline (dose and route of administration not provided) exhibited anticarcinogenic activity toward P-388 lymphocytic leukemia in mice (strain not provided) (Agarwal and Rastogi, 1975; cited by Anthoni et al., 1991).

Table 3. Short-term and Subchronic Exposure to Trigonelline

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/Observation Period	Results/Comments	Reference
albino Sabra rats, 21-day-old	12F	trigonelline, purity n.p.	50 mg/kg/day (0.36 mmol/kg/day), orally	21 day exposure; sacrificed after 42 days	After sacrifice, the liver, kidney, thymus, thyroid, adrenals, uterus, and ovaries were removed and weighed. No changes in organ weights were observed in the trigonelline-treated group. No animals died.	Shani (Mishkinsky) et al. (1974)
cats, strain and age n.p.	n.p.	trigonelline, purity n.p.	3,500 mg/day (26 mmol/day)	62-70 day exposure	No visible effects were noted.	Faulkner and Smith (1950)

Table 4. Anticarcinogenicity of Trigonelline

Species, Strain, and Age	Number and Sex of Animals	Chemical Form and Purity	Dose	Exposure/Observation Period	Results/Comments	References
mice (strain and age n.p.)	n.p.	trigonelline isolated from <i>Mappia foetida</i>	n.p.	n.p.	Exhibited anticarcinogenic activity against P-388 lymphocytic leukemia.	Agarwal and Rastogi (1975; cited by Anthoni et al., 1991)

Abbreviations: n.p. = not provided

9.5 Genotoxicity

These studies are presented in **Table 5**.

9.5.1 Prokaryotic Systems

Trigonelline was not mutagenic at concentrations up to 10,000 µg/plate (73 µmol/plate) in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence or absence of metabolic activation (Fung et al., 1988).

In a study designed to mimick coffee roasting, 1000 µmol of a thermal decomposition product of trigonelline (prepared by heating at 250°C for 20 min.) was mutagenic in *S. typhimurium* strain TA 98 in the presence of metabolic activation (Wu et al., 1997). Trigonelline (1000 µmol) was also mutagenic in the same strain in the presence of metabolic activation when heated at 250°C for 20 min. in combination with alanine, arginine, cysteine, lysine, phenylalanine, proline, serine, threonine, valine, or glucose. When trigonelline (1000 µmol) was heated (as previously stated) in combination with a mix of amino acids and glucose, the reaction products were mutagenic in *S. typhimurium* strains TA98 and YG1024 but not in strain YG1029 in the presence of metabolic activation. In the absence of S9, the reaction products were mutagenic in strains TA98 and YG1029, but were toxic to YG1024.

9.5.2 *In vitro* Mammalian Systems

Trigonelline was not mutagenic at concentrations up to 7400 µg/plate (54 µmol/plate) in the L5178Y TK^{+/−} mouse lymphoma mutation assay, with and without metabolic activation (Fung et al., 1988).

9.6 Immunotoxicity

No data were found.

9.7 Other Data

9.7.1 Hypocholesterolemic Activity

Trigonelline (dose and route of administration not provided) reduced the total and the free plasma cholesterol levels in rats (strain not provided) (Abe and Kaneda, 1975).

9.7.2 Hypoglycemic Activity

Trigonelline counteracted the hyperglycemic effect of cortisone when administered concomitantly (dose and route of administration not provided), but not when administered 2 hours after cortisone administration to non-diabetic rabbits (Menczel and Sulman, 1962; Menczel et al., 1964; 1965; Shani (Mishkinsky) et al., 1973; all cited by Shani (Mishkinsky) et al., 1974).

Table 5. Genotoxicity of Trigonelline

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
9.5.1 Prokaryotic Systems							
<i>Salmonella typhimurium</i> strains TA1535, TA1537, TA1538, TA98, and TA100	<i>his</i> gene mutations	+/-	trigonelline, purity n.p.	up to 10,000 µg/plate (73 µmol/plate)	negative		Fung et al. (1988)
<i>S. typhimurium</i> strain TA98	<i>his</i> gene mutations	+	trigonelline, >99% purity, as a thermal decomposition product	1000 µmol	positive	Thermal decomposition product prepared by heating at 250°C for 20 min. Trigonelline had the highest mutagenic activity of the 13 compounds tested.	Wu et al. (1997)
				1000 µmol trigonelline plus 1000 µmol of alanine, arginine, cysteine, cystine, lysine, phenylalanine, proline, serine, threonine, tryptophan, or valine, or 200,000 µM glucose	positive when trigonelline was heated in combination with alanine, arginine, cysteine, lysine, phenylalanine, proline, serine, threonine, valine, or glucose negative when in combination with cystine or tryptophan	The binary combinations were heated at 250°C for 20 min. Highest levels of mutagenic activity were found for the binary combinations of trigonelline with serine or threonine.	

Table 5. Genotoxicity of Trigonelline (continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
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Abbreviations: n.p. = not provided

Table 5. Genotoxicity of Trigonelline (continued)

Test System	Biological Endpoint	S9 Metabolic Activation	Chemical Form, Purity	Dose	Endpoint Response	Comments	Reference
<i>S. typhimurium</i> strains TA98, YG1024, and YG1029	<i>his</i> gene mutations	+/-	trigonelline, >99% purity, as a thermal decomposition product	1000 µmol trigonelline plus amino acid mixtures (1000 or 350 µmol) and glucose	positive (TA98 with and without S9; YG1024 with S9; and YG1029 without S9) negative (YG1029 with S9) toxic (YG1024 without S9)	The combinations were heated at 250°C for 20 min.	Wu et al. (1997) (cont.)
9.5.2 In vitro Mammalian Systems							
L5178Y mouse lymphoma cells	<i>TK</i> ^{+/−} gene mutations	+/-	trigonelline, purity n.p.	up to 7400 µg/mL (54 µmol/mL)	negative		Fung et al. (1988)

Abbreviations: n.p. = not provided

In Sabra albino rats with alloxan-induced diabetes, trigonelline exhibited a mild and transient hypoglycemic effect (Shani (Mishkinsky) et al., 1974). Rats were given 250 or 1000 mg/kg (1.8 or 7.3 mmol/kg) trigonelline via a stomach tube or in the drinking water.

9.7.3 Inhibition of Glycine Betaine Accumulation in Kidney Cells *In Vitro*

High concentrations (250 μmol) of trigonelline, added under hypertonic conditions, inhibited glycine betaine $[(\text{CH}_3)_3\text{N}^+\text{CH}_2\text{CO}_2^-]$ accumulation in osmotically stressed Madin Darby canine kidney cells *in vitro* (Randall et al., 1996); glycine betaine, accumulated in the inner medulla of the kidney, is believed to balance the hyperosmotic environment and to counter urea denaturation in mammals. Trigonelline is a betaine molecule with a quaternary ammonium moiety (R_4N^+) and a carboxylate moiety ($-\text{CO}_2^-$).

9.7.4 Effects of Trigonelline in Plants

Trigonelline is involved in G2 cell cycle arrest (Evans et al., 1979; Evans and Tramontano, 1981; both cited by Tramontano et al., 1986; Lynn et al., 1978; Nakanishi, 1979) in both roots and shoots of plants after seed germination (Lynn et al., 1978). Additionally, nicotinamide and trigonelline may function as signal transmitters in the response to oxidative stress in plants since UV-B and oxidative stress induced an increase in nicotinamide and trigonelline contents in plant tissues *in vitro* (Berglund et al. 1996). Poly(ADP-ribose) is thought to be involved in the mechanism of cell arrest (Tramontano et al., 1990) and in the induction of defensive metabolism. The physiological effects of trigonelline are thought to occur at the level of DNA methylation, probably influencing the accessibility of DNA for transcription (Klaas et al., 1989; cited by Berglund, 1994).

10.0 STRUCTURE-ACTIVITY RELATIONSHIPS

Various *N'*-methylnicotinamide analogs inhibit *N'*-methylnicotinamide uptake by the rat kidney cortex (Reynard, 1968). Unsubstituted *N*-methylpyridine produced considerable inhibition, while the introduction of a carboxamide group at positions 2 and 4 on the pyridine ring

lowered the inhibitory capacity. Marked inhibition was produced by 1-methyl-2-acetylpyridine and 1-methyl-4-acetylpyridine. Trigonelline was not inhibitory, which the authors attributed to the negative charge of the carboxyl group. Methyl *N*-methylpyridine-3-carboxylate, in which the negative charge was eliminated, was inhibitory. The methyl, ethyl, and butyl esters of trigonelline all produced strong inhibition.

Niacin, nicotinamide, and some of their precursors, analogs, and derivatives are active alleviating agents for dicrotophos-induced teratogenesis (Roger et al., 1969). The active niacin analogs are those that may be converted to niacin or nicotinamide, while the inactive analogs (e.g., trigonelline) are those that are not likely to be converted to niacin or nicotinamide.

11.0 ONLINE DATABASES AND SECONDARY REFERENCES

11.1 Online Databases

Chemical Information System Files

SANSS

TSCATS (Toxic Substances Control Act Test Submissions)

DIALOG Files

Chem. Econ. Hdbk.

NIOSHTIC

KIRK-OTHMER ENCYCLOPEDIA OF CHEM. TECHNOL.

National Library of Medicine Databases

EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

AGRICOLA

BIOSIS

CA File

CABA

CANCERLIT

CAPLUS

CBNB

CEN

CHEMLIST

CROPB

CROPU

CSNB

DDFB

DDFU

DRUGLAUNCH

EMBASE

FSTA

HODOC

IPA

LIFESCI

MEDLINE

NAPRALERT
PHIN

PROMPT
RTECS

TOXLINE
TOXLIT

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC
Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSH TIC7	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

11.2 Secondary References

CRC Handbook of Chemistry and Physics, Weast, R.C., and M.J. Astle, Eds. CRC Press, Boca Raton, FL, 1980.

The Merck Index, 12th ed., Budavari, S. Ed. Merck & Co., Inc., Whitehall, NJ. Listed in Section 12 as Budavari (1996).

12.0 REFERENCES

Abe, S. and T. Kaneda. 1975. Effect of betaines and taurine and its derivatives on plasma cholesterol levels in rats. *Eiyo To Shokuryo* 28(3):125-128. In Japanese (except abstract in English).

Anonymous. 1983. New hope for Parkinsonism. *Chem. Week* (27 April 1983):331.

Anthoni, U., C. Christophersen, L. Hougaard, and P. H. Nielsen. 1991. Review: Quaternary ammonium compounds in the biosphere—an example of a versatile adaptive strategy. *Comp. Biochem. Physiol.* 99B(1):1-18.

Beckstrom-Sternberg and J.A. Duke. 1997. Searches for plants containing trigonelline and biological activities of trigonelline. *Phytochemeco Database* produced by USDA Agricultural Research Service. Internet URL:<http://sun.ars-grin.gov/cgi-bin/duke/ethnobot.pl>.

Berglund, T. 1994. Nicotinamide, a missing link in the early stress response in eukaryotic cells: a hypothesis with special reference to oxidative stress in plants. *FEBS Lett.* 351:145-149.

Berglund, T., G. Kalbin, A. Strid, J. Rydström, A. B. Ohlsson. 1996. UV-B- and oxidative stress-induced increase in nicotinamide and trigonelline and inhibition of defensive metabolism induction by poly(ADP-ribose)polymerase inhibitor in plant tissue. *FEBS Lett.* 380:188-193.

Bodor, N. and H. H. Farag. 1983. Improved delivery through biological membranes. II. A redox chemical drug-delivery system and its use for brain-specific delivery of phenylethylamine. *J. Med. Chem.* 26:313-318.

Budavari, S. Ed. 1996. Trigonelline. In: *The Merck Index*. 12th ed., Merck & Co., Inc., Whitehall, NJ. p. 1651.

Carter, E. G. A. and K. J. Carpenter. 1981. Trigonelline not a metabolite of bound niacin from cereals. *Fed. Proc.* 40:3II. No. 3604.

Chemlist. 1997. Database search for trigonelline. *Chemlist Abstract No.* 109540.

Chikhale, P. and N. Bodor. 1991. Improved delivery of acyclovir to the skin using a dihydrotrigonelline trigonelline redox carrier. *J. Pharm. Sci.* 80(4):402-403.

Czok, G. 1974. Biopharmacological effects of coffee substances other than caffeine. *Arch. Sci. Med.* 131(1):15-17.

Der Marderosian, A. H. 1977. Pharmacognosy: Medicinal teas—boon or bane? *Drug Ther.* 7 (Research Paper):178-186.

Faulkner, K. K. and J. W. H. Smith. 1950. Preliminary studies of the toxicity of locoweed extracts. *Proc. Oklahoma Acad. Sci.* 31:48-50.

Fung, V. A., T. P. Cameron, T. J. Hughes, P. E. Kirby, and V. C. Dunkel. 1988. Mutagenic activity of some coffee flavor ingredients. *Mutat. Res.* 204:219-228.

- Gill, E. W., W. D. M. Paton, and R. G. Pertwee. 1970. Preliminary experiments on the chemistry and pharmacology of Cannabis. *Nature (London)* 228(Oct. 10):134-136.
- Höhl, U., B. Upmeier, and W. Barz. 1988. Growth and nicotinate biotransformation in batch cultured and airlift fermenter grown soybean cell suspension cultures. *Appl. Microbiol. Biotechnol.* 28:319-323.
- IARC (International Agency for Research on Cancer). 1991. Coffee, Tea, Mate, Methylxanthines, and Methylglyoxal. In: *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans*. Vol. 51:69.
- Ito, Y., T. Suzuki, T. Shirai, and T. Hirano. 1994. Presence of cyclic betaines in fish. *Comp. Biochem. Physiol.* 199B(1):115-124.
- Li, C. P. 1974. *Chinese Herbal Medicine*, NIH Pub. No. 75-732. U.S. Dept. of Health, Education, and Welfare, Washington, D.C.
- Lynn, D. G., K. Nakanishi, S. L. Patt, J. L. Occolowitz, S. Almeida, and L. S. Evans. 1978. Isolation and characterization of the first mitotic cycle hormone that regulates cell proliferation. *J. Am. Chem. Soc.* 100:7759.
- Mai, J. and H. Mai. 1997. Oral preparations containing fenugreek extracts for strengthening nail and hair. U.S Patent No. 95-541421 from CAPLUS 1997:351136.
- Nakanishi, K. 1979. Studies on some biologically active compounds. *Pure Appl. Chem.* 51:731-745.
- Palomino, E., D. Kessel, and J. P. Horwitz. 1989. A dihydropyridine carrier system for sustained delivery of 2',3'-dideoxynucleosides to the brain. *J. Med. Chem.* 32:622-625.
- Physicians' Desk Reference. 1995. Nicolar. pp. 1973-1975.
- Pop, E., E. Shek, T. Murakami, and N. S. Bodor. 1989. Improved anticonvulsant activity of phenytoin by a redox brain delivery system I: Synthesis and some properties of the dihydropyridine derivatives. *J. Pharm. Sci.* 78(8):609-616.
- Randall, K., M. Lever, B. A. Peddie, and S. T. Chambers. 1996. Accumulation of natural and synthetic betaines by a mammalian renal cell line. *Biochem. Cell Biol.* 74:283-287.
- Reynard, A. M. 1968. The reversible and irreversible inhibition of *N*'-methylnicotinamide uptake into rat kidney cortex slices. *J. Pharmacol. Exp. Ther.* 163(2):461-467.

Roger, J.-C., D. G. Upshall, and J. E. Casida. 1969. Structure-activity and metabolism studies on organophosphate teratogens and their alleviating agents in developing hen eggs with special emphasis on bidrin. *Biochem. Pharmacol.* 18:373-392.

Shani (Mishkinsky), J., A. Goldschmied, B. Joseph, Z. Ahronson, and F. G. Sulman. 1974. Hypoglycaemic effect of *Trigonella Foenum Graecum* [sic] and *Lupinus Termis* (Leguminosae) seeds and their major alkaloids in alloxan-diabetic and normal rats. *Arch. Int. Pharmacodyn.* 210:27-37.

Shibata, K. and H. Taguchi. 1987. Effect of dietary *N'*-methylnicotinamide or trigonelline on the growth and niacin metabolism in weanling rats. *Vitamins* 61(10):493-499. Abstract from CAPLUS 1988:36568.

Taguchi, H., M. Sakaguchi, and Y. Shimabayashi. 1985. Trigonelline content in coffee beans and the thermal conversion of trigonelline into nicotinic acid during the roasting of coffee beans. *Agric. Biol. Chem.* 49(12):3467-3472. Abstract from BIOSIS 86:238715.

Taguchi, H., M. Sakaguchi, and Y. Shimabayashi. 1986. Contents of quinolinic acid trigonelline and *N*-1 methylnicotinamide in various foods and thermal conversion of these compounds into nicotinic acid and nicotinamide. *Vitamins* 60(11):537-546. Abstract from BIOSIS 87:88133

Tramontano, W. A., P. A. McGinley, E. F. Ciancaglini, and L. S. Evans. 1986. A survey of trigonelline concentrations in dry seeds of the Dicotyledoneae. *Environ. Exp. Bot.* 26(3):197-205.

Tramontano, W. A., D. A. Phillips, C. A. Carman, and A. M. Massaro. 1990. Nuclear incorporation of [adenine-¹⁴C]NAD is altered by compounds which affect poly(ADP-ribose) formation. *Phytochemistry* 29(1):31-33.

Viani, R. and I. Horman. 1974. Thermal behavior of trigonelline. *J. Food Sci.* 39(6):1216-1217.

Wasserman, G., H. D. Stahl, W. Rehman, and P. Whitman. 1993. Coffee. In: *Kirk-Othmer Encyclopedia of Chemical Technology*, 4th ed., Vol. 6. John Wiley and Sons, New York.

Wu, X., K. Skog, and M. Jägerstad. 1997. Trigonelline, a naturally occurring constituent of green coffee beans behind the mutagenic activity of roasted coffee? *Mutat. Res.* 391:171-177.

Yuyama, S. and Y. Kawano. 1996. Urinary excretion of *N'*-methyl-2-pyridone-5-carboxylic acid and the fate of remaining of trigonelline. *Adv. Exp. Med. Biol.* 398:599-603.

Yuyama, S. and T. Suzuki. 1991. The excretion of *N'*-methyl-2-pyridone-5-carboxylic acid and related compounds in human subjects after oral administration of nicotinic acid, trigonelline, and *N'*-methyl-2-pyridone-5-carboxylic acid. *Adv. Exp. Med. Biol.* 294:475-479.

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