Tutin: Its Pharmacological Action and Its Antagonism with Sodium Amytal*

By Edward E. Swanson

Several species of the genus *Coriaria* are known to be poisonous to livestock and men. In a previous communication (1), we reported the isolation and pharmacological action of coriamyrtin, a toxic principle from the leaves and stems of *Coriaria myrtifolia*. This species grows in Southern France and other countries bordering the Mediterranean Sea. Maloney (2), using our material, made a similar investigation of coriamyrtin. In 1900, Easterfield and Aston (3) isolated a toxic glucoside named tutin from three *Coriaria* species, *C. thymofolia*, *C. ruscifolia* and *C. augustissima*. These plants are indigenous to New Zealand. The pharmacological action of tutin has been reported by Easterfield and Aston (3), (4), Fitchett and Malcohm (5), Christie (6) and Marshall (7). Their results indicate that it is a convulsant drug similar to picrotoxin and coriamyrtin.

Our interest in coriamyrtin led us to extend our studies with tutin. Through the generous coöperation of Dr. B. C. Aston, Chief Chemist of the New Zealand Department of Agriculture, Wellington, New Zealand, we were in possession of a shipment of *Coriaria thymofolia* which he personally collected and dried. In addition, Dr. Aston was kind enough to send us a sample of his impure tutin which could be easily purified and recrystallized.

EXPERIMENTAL

Isolation of Tutin.—The isolation of tutin was achieved by percolating the pulverized plant with ethyl alcohol. Upon evaporation, the syrup was diluted with water. Tutin was then extracted with ether from the aqueous solution, and it was finally crystallized from ethyl alcohol. The pure substance, in oblique-ended prisms, is more soluble in ether than chloroform, soluble in hot alcohol, less soluble in cold alcohol and slightly soluble in water. It melts at 215–216° C. (corrected) and has a specific rotation $[\alpha]_D^{25} = +50°$ in chloroform. Analytical data and molecular weight determinations suggest the empirical formula, $C_{20}H_{24}O_8$ (calculated C 61.35, H 5.93, mol. wt. 391; found C 61.36, 61.32, H 6.03, 5.96, mol. wt. 418, 421). Easterfield and Aston (3) reported the empirical formula, $C_{17}H_{20}O_7$ (calculated C 60.7, H 5.9, mol. wt. 336; found C 60.8, H 6.0, mol. wt. 334, 327), with a melting point of 208–209° C. Tutin when boiled with dilute hydrochloric acid reduces Fehling's solution, thus confirming that it is a glucoside (glycoside). For pharmacological experiments, a stock solution of 1:1000 tutin in approximately 10 per cent alcohol was employed, and necessary dilutions were made from it when needed.

Convulsions, Toxicity and Other Effects of Tutin.—When injected into the abdominal lymph sac of frogs, tutin in the dosage of 0.002 mg. per Gm. or more caused convulsions in approximately 30–50 minutes. Coriamyrtin (1) produced convulsions in 13 minutes with 0.001 mg. per Gm. by the same route of administration. Thus, in frogs tutin is considerably slower acting than coriamyrtin. The convulsions of tutin were clonic in type, with head drawn back and legs extended. Tutin convulsions were less severe and less frequent than those induced by coriamyrtin.

As with coriamyrtin (1), the origin of convulsions due to tutin is also apparently in the medulla and upper cord, because decapitation in frogs did not stop the convulsion, whereas destruction of the medulla and upper cord abolished their further occurrence.

In rats and rabbits, tutin in sufficient amounts, given either intravenously or subcutaneously, produced clonic convulsions, with the head retracted and legs extended. The animals, particularly rabbits, assumed an opisthotonic posture. External stimuli did not appear to have much influence upon the onset of convulsions.

Comparisons were made with other convulsants—coriamyrtin, picrotoxin, metrazol and thujone.

Table I summarizes the minimal convulsive doses (M. C. D.), the time of onset of convulsions and the minimal lethal doses (M. L. D.) in mice and rabbits following intravenous and subcutaneous injections. From the same data, the median convulsive dose (CD_{50} ± Standard Error) and the median lethal dose (LD_{50} ± S. E.) could also be computed according to the formula of Bliss (8). The ratio between M. L. D. and M. C. D. varies according to the drug as well as the mode of administration.

As also shown in Table I, coriamyrtin has a most prompt action following an M. C. D., while tutin requires the longest latent period preceding the onset of convulsions. The remaining drugs, picrotoxin, metrazol and thujone, have an intermediate speed in the development of convulsions. When the animals survived, recovery after tutin took place more slowly than after other convulsants studied.

Tutin was found to increase the amplitude and rate of respiration, raise the blood pressure, slightly accelerate the heart rate, have very slight effect on isolated rabbits' uteri and intestines, and cause no

* From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana.

changes in the caliber of frogs' leg vessels by perfusion with a 1:100,000 solution. These results are comparable to those with coriamyrtin as reported previously (1).

took place. Rabbits were used throughout the experiments.

From Table II, it should be noted that "Sodium Amytal" saved 4 out of 5 animals receiving 55

TABLE I.—TOXICITY OF TUTIN IN COMPARISON WITH CORIAMYRTIN, PICROTOXIN, METRAZOL AND THUJONE

Compound	Species of Animals	Number of Animals Used	Method of Administration	Observed M. C. D. Mg. per Kg.	Observed M. L. D. Mg. per Kg.	Onset of Convulsions after M. C. D., Minutes	Computed CD_{50} ± S. E. Mg. per Kg.	Computed LD_{50} ± S. E. Mg. per Kg.
Tutin	Mice	153	Subcutaneous	1.60	4.00	35	1.226 ±0.202	3.613 ±0.309
	Rabbits	39	Subcutaneous	0.80	1.50	79	0.709 ±0.040	1.521 ±0.073
		30	Intravenous	0.50	1.25	46	0.448 ±0.034	1.244 ±0.100
Coriamyrtin	Mice	89	Subcutaneous	0.90	3.50	9	0.816 ±0.048	3.234 ±0.261
	Rabbits	30	Subcutaneous	0.30	1.20	10	0.291 ±0.024	0.930 ±0.045
		48	Intravenous	0.14	0.40	1−	0.129 ±0.006	0.371 ±0.026
Picrotoxin	Mice	90	Subcutaneous	3.00	11.00	20	2.815 ±0.267	10.52 ±0.920
	Rabbits	45	Subcutaneous	2.00	2.50	48	1.098 ±0.154	1.956 ±0.176
		48	Intravenous	0.50	1.25	20	0.450 ±0.048	1.016 ±0.059
Metrazol	Mice	175	Subcutaneous	60.0	114.0	18	58.80 ±2.310	110.10 ±3.050
	Rabbits	33	Subcutaneous	50.0	87.5	15	44.82 ±5.44	76.00 ±3.390
		38	Intravenous	11.0	80.0	1+	10.43 ±0.41	74.73 ±2.460
Thujone	Mice	45	Subcutaneous	1.25 cc.	3.00 cc.	18	1.094 ±0.109	2.157 ±0.331
	Rabbits	50	Subcutaneous	0.30 cc.	0.40 cc.	16	0.181 ±0.043	0.362 ±0.070
		52	Intravenous	0.003 cc.	0.035 cc.	1	0.00235 ±0.00031	0.031 ±0.001

Detoxification of Tutin in Comparison with Coriamyrtin, Picrotoxin, Metrazol and Thujone by "Sodium Amytal."—In view of the fact that picrotoxin and coriamyrtin (1) are detoxified by certain barbiturates, it might be expected that "Sodium Amytal" (Sodium Iso-amyl Ethyl Barbiturate, Lilly) could combat the excessive doses of tutin, metrazol and thujone. The same technique, first elaborated by Barlow (9) for strychnine poisoning, was adopted in the present investigation; that is, the convulsant substances were injected subcutaneously, and "Sodium Amytal" was administered by vein and repeated when recurrence of convulsions

M. L. D.'s of tutin (75 mg. per Kg.), but none of the rabbits was saved with 60 M. L. D.'s. As previously shown (1), 45 M. L. D.'s of coriamyrtin and 40 M. L. D.'s of picrotoxin were detoxified by "Sodium Amytal." Thus, more lethal doses of tutin were antidoted by "Sodium Amytal" than with coriamyrtin or picrotoxin. It may be pointed out that in tutin poisoning more "Sodium Amytal" was needed in order to obtain successful results, which is undoubtedly due to its protracted course of intoxication. The practical application of this antagonism to the treatment of poisoning by *Coriaria thymofolia* with "Sodium Amytal" is only obvious.

TABLE II.—DETOXIFICATION OF TUTIN BY "SODIUM AMYTAL" IN COMPARISON WITH OTHER CONVULSANTS IN RABBITS

Drug	Quantity Given Subcutaneously, Mg. per Kg.	Equivalent to Number of M. L. D.'s	Average Amount of "Sodium Amytal" Injected Intravenously, Mg. per Kg.	Number of Animals Used	Number of Animals Died
Tutin	52.5	35	508.30	3	0
	60.0	40	575.00	3	0
	67.5	45	616.60	3	0
	75.0	50	625.00	3	0
	82.5	55	650.00	5	1
	90.0	60	533.30	3	3
Coriamyrtin	48.0	40	129.2	3	0
	54.0	45	132.5	5	2
	60.0	50	145.8	3	3
Picrotoxin	87.5	35	153.0	5	0
	100.0	40	210.0	5	1
	112.5	45	234.5	5	4
Metrazol	218.75	2½	75.0	5	0
	437.50	5	150.0	5	0
	656.25	7½	225.0	5	2
	875.00	10	250.0	3	3
Thujone	1.0 cc.	2½	40.19	5	0
	2.0 cc.	5	45.00	5	1
	3.0 cc.	7½	57.75	5	4
	4.0 cc.	10	60.20	5	5

In the detoxification of metrazol and thujone, the results were not so successful as with tutin, coriamyrtin or picrotoxin. As shown in Table II, only $7^1/_2$ M. L. D.'s of metrazol and 5 M. L. D.'s of thujone could be tolerated with the help of "Sodium Amytal." According to Fischer and Löwenback (10), metrazol has a pronounced effect on the cerebrum. Sparks (11) found that thujone also acts on the cortex. Picrotoxin, coriamyrtin and tutin, all have an action on the medulla. It appears that "Sodium Amytal" is more effective in combating convulsants acting upon the medulla than those acting upon the brain.

Detoxification of "Sodium Amytal" by Tutin.—In view of the fact that there is a mutual antagonism between certain convulsants and barbiturates, it might be expected that tutin could detoxify lethal doses of barbiturates, such as "Sodium Amytal." In barbiturate poisoning, the value of picrotoxin has been repeatedly demonstrated by Maloney (12), (13), (14) and Koppanyi and his associates (15). In a previous communication, we showed that in rabbits coriamyrtin or picrotoxin can detoxify $1^3/_4$ but not 2 M. L. D.'s of "Sodium Amytal." With coriamyrtin Maloney (2) reported a similar action against three different barbiturates. In the present study, "Sodium Amytal" was injected subcutaneously rather than intraperitoneally, as previously administered (1), because of the slow onset of action of tutin. Repeated doses of tutin were given by vein to keep the respiration stimulated and reflexes active.

TABLE III. DETOXIFICATION OF "SODIUM AMYTAL" BY TUTIN IN RABBITS

Quantity of "Sodium Amytal" Subcutaneously, Mg. per Kg.	Equivalent to Number of M. L. D.'s of "Sodium Amytal"	Average Quantity of Antidote by Vein, Mg. per Kg.	Number of Animals Used	Number of Animals Died
150	1	6.25	1	0
200	$1^1/_3$	9.375	3	0
250	$1^2/_3$	15.625	3	0
300	2	19.52	3	0
350	$2^1/_3$	23.43	3	1
400	$2^2/_3$	20.00	3	3

As shown in Table III, tutin saved all animals receiving 1 and 2 M. L. D.'s of "Sodium Amytal." Two out of 3 also recovered from $2^1/_3$ M. L. D.'s of "Sodium Amytal" by tutin treatment, but all 3 animals died from $2^2/_3$ M. L. D.'s. It is very obvious from the above results that "Sodium Amytal" is far more effective in antidoting tutin than vice versa.

SUMMARY

1. Tutin, $C_{20}H_{23}O_8$, a glycoside of *Coriaria thymofolia*, has a convulsant action similar to that of coriamyrtin and picrotoxin. It is slower in the onset of action but longer in duration.

2. Comparisons have been made with coriamyrtin, picrotoxin, metrazol and thujone, in mice and rabbits, by the determination of convulsive and lethal doses, injected subcutaneously and intravenously.

3. In rabbits "Sodium Amytal" can detoxify 55 minimal lethal doses of tutin, 45 minimal lethal doses of coriamyrtin, 40 minimal lethal doses of picrotoxin, but only 7.5 minimal lethal doses of metrazol and 5 minimal lethal doses of thujone.

4. Conversely, tutin given by vein can detoxify in rabbits a little more than 2 minimal lethal doses of "Sodium Amytal" injected subcutaneously.

The author is indebted to Dr. K. K. Chen for his valuable suggestions during the investigation and his assistance in preparation of the manuscript. Thanks are also due to Messrs. W. E. Fry, E. Brown Robbins and Herbert Glick for their technical assistance in this work.

REFERENCES

(1) Swanson, *J. Pharmacol. & Exper. Therap.*, 57, 410 (1936).

(2) Maloney, *Ibid.*, 57, 361 (1936).

(3) Easterfield and Aston, *New Zealand Dept. of Agri.*, Part I, 8 (1900).

(4) Easterfield and Aston, *Ibid.*, Part II, 4 (1901).

(5) Fitchett and Malcohm, *Quart. J. Exper. Physiol.*, 2, 335 (1909).

(6) Christie, *New Zealand Med. J.*, 3, 211 (1889–1890); 4, 1 (1890–1891).

(7) Marshall, *J. Pharmacol. & Exper. Therap.*, 4, 135 (1912–1913).

(8) Bliss, *Quart. J. Pharm. & Pharmacol.*, 11, 192 (1938).

(9) Barlow, *J. A. M. A.*, 98, 1980 (1932).

(10) Fischer and Löwenback, *Arch. exptl. Path. Pharmakol.*, 174, 502 (1934).

(11) Sparks, *Arch. internat. de pharmacodyn. et de thérapie*, 33, 460 (1927).

(12) Maloney, Fitch and Tatum, *J. Pharmacol. & Exper. Therap.*, 41, 465 (1931).

(13) Maloney and Tatum, *Ibid.*, 44, 337 (1932).

(14) Maloney, *Ibid.*, 49, 133 (1933); *J. Nat. M. A.*, 25, 47, 90 (1933).

(15) Linegar, Dille and Koppanyi, *Science*, 82, 376 (1935); *Proc. Soc. Exptl. Biol. & Med.*, 33, 396 (1935–1936).