methanol. The attack of a proton on the oxygen atom of the amino-methanol followed by expulsion of water leads to a resonance established carbonium-immonium ion. This electrophilic carbonium ion then reacts with a nucleophile which, under Mannich conditions, is usually the carbanion resulting from the ionization of the active hydrogen containing compound. It appears that under Mannich conditions AZBN acts as the nucleophile as well as condensing with the formaldehyde.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The infrared spectra were charted on a Perkin Elmer model 137 spectrophotometer in Nujol mull. The NMR spectrum was recorded with a Varian A-60 spectrometer in $CDCl_3$, using tetramethylsilane as the internal standard.

Attempted Mannich Condensation of Indanedione-1,3 with AZBN.—AZBN (12.51 Gm., 0.10 mole) dissolved in 50 ml. of ethanol was acidified to pH 4 by dropwise addition of concentrated hydrochloric acid. Indanedione (14.6 Gm., 0.10 mole) was added followed by 4.5 Gm. (0.15 mole) of paraformaldehyde. The reaction mixture was heated on a water bath for 4 hr. The reaction mixture was cooled and filtered. This gave a product weighing 11.5 Gm. which could not be recrystallized because of its insolubility in most organic solvents. The mother liquor was diluted with 100 ml. of acetone and refrigerated overnight. A crystalline solid (6.5 Gm.) was obtained; this was recrystallized from ethanol, m.p. 301° dec. NMR data, δ = 1.95 (16H, singlet); 2.17 (6H, multiplet); 3.36 (8H, triplet); 7.47 (2H, singlet).

Anal.—Calcd. for $C_{17}H_{32}Cl_2N_2$: C, 60.90; H, 9.59; Cl, 21.74; N, 8.35. Found: C, 60.13; H, 9.77; Cl, 21.81; N, 8.55.

Methylenebis-3-azabicyclo(3.2.2)nonane Dihydrochloride (I).—AZBN (6.25 Gm., 0.05 mole) dissolved in 25 ml. of ethanol was acidified with concentrated hydrochloric acid to pH 4. Paraformaldehyde (1.11 Gm., 0.037 mole) was added and the reaction mixture refluxed on a boiling water bath for 3 hr. At the end of this period the contents were cooled and 100 ml. of acetone was added. The solution was refrigerated overnight. The desired product which was obtained as white needles, was recrystallized from ethanol or ethanol–acetone, m.p. 301° dec. Yield, 5.5 Gm. (65%). The mixed melting point obtained with the product isolated from the Mannich condensation (AZBN) showed no depression. Infrared spectra of both products were identical.

Anal.—Calcd. for $C_{17}H_{32}Cl_2N_2$: N, 8.35; Found: N, 8.53.

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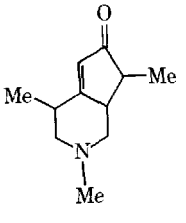
Antidiabetic Effect of Tecomine and Tecostanine

By YOUSSEF HAMMOUDA and M. SAMIR AMER*

The hypoglycemic properties of tecomine citrate and tecostanine hydrochloride on fasting blood sugar, glucose tolerance, depancreatized, and alloxan-diabetic rabbits is described. The two drugs proved to be effective antidiabetic agents only in the presence of the pancreas.

TECOMINE (I) and tecostanine (II) are two alkaloids isolated by Hammouda and Motawi (1) and Hammouda *et al.* (2) from the leaves of *Tecoma stans* (Juss.). The leaves of the various species of *Tecoma* have long been used by the natives in Mexico for the control of diabetes (3, 4). Since the structure of the two alkaloids isolated therefrom was elucidated (5–7), it was of interest to determine whether the two alkaloids are responsible for the long known antidiabetic properties of the leaves. The present study was initiated to determine the hypoglycemic properties of the two alkaloids and to determine the possible mechanism by which they produce this effect. A short note was previously published on this subject (8).



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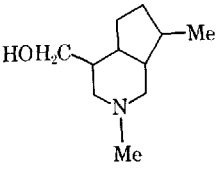

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TABLE 1.—EFFECT OF TECOMINE CITRATE AND TECOSTANINE HYDROCHLORIDE ON THE FASTING SUGAR LEVEL IN RABBITS

Time After Injection, hr.	Blood Sugar Level, mg./100 ml. Blood[a]				
	Tecomine Citrate		Tecostanine HCl		Tolbutamide
	Oral, 50 mg.[b]/Kg.	i.v., 20 mg.[b]/Kg.	Oral, 50 mg.[b]/Kg.	i.v., 20 mg.[b]/Kg.	Oral, 250 mg./Kg.
0	107.3	98.3	110.0	104.9	100.1
1	100.1	74.3	111.0	83.4	99.1
2	101.3	61.5	102.6	67.7	86.3
3	83.6	55.3	75.3	50.3	79.1
4	64.5	60.7	54.3	59.5	78.6
5	49.7	73.3	59.6	70.1	83.3
6	53.6	80.2	81.1	79.7	89.5
10	92.7	99.3	97.3	101.9	93.3
24	100.7	90.1	95.5	102.3	105.1

[a] Average of four to six experiments. [b] Calculated as the free base.

MATERIALS AND METHODS

The animals used in the present study were healthy male rabbits weighing 1.5–2.5 Kg. fed *ad libitum* on a balanced diet. Blood samples for the assay of blood sugar were obtained by bleeding the ear vein into a heparinized pipet. Blood sugar was determined by the method of Nelson (9). Alloxan diabetes was produced by injecting alloxan monohydrate[1] intravenously in a dose of 200 mg./Kg. in 4 divided doses over a period of 24 hr. Alloxan was dissolved in isotonic saline to make a 3% solution immediately before injection. The animals were allowed to drink a 5% glucose solution for the entire period of the alloxan diabetes. Hyperglycemia developed slowly and reached a maximum after 2 weeks. The animals were used only after producing glucosuria continuously for 3 consecutive days. Tecomine citrate and tecostanine hydrochloride were prepared from the dried fresh leaves of *T. stans* (1) and were tested for purity before use.

RESULTS

Fasting Animals.—The rabbits used in this study were fasted for 12–18 hr. before the experiments were started. The drugs were dissolved in physiological saline and given either intravenously or orally *via* a stomach tube. The results are given in Table I.

It is clear from the table that both alkaloids are potent hypoglycemic agents producing severe reductions in the blood sugar of fasting rabbits when given in a dose of 20 mg./Kg. i.v. or 50 mg./Kg. orally. When given intravenously the maximum hypoglycemic effect is reached earlier than when given orally even in higher doses. This is to be expected since the drugs have to be absorbed from the alimentary tract. The absorption from the alimentary tract would account for the 1–2-hr. lag when the drug is given orally. When given intravenously, the effect on the blood sugar reaches maximum (179% of tolbutamide) after 3 hr. and 14 min. for tecomine citrate and after 3 hr. and 23 min. for tecostanine hydrochloride (186% of tolbutamide). Some of the animals experienced hypoglycemic coma at the height of the hypoglycemic effect.

Glucose Tolerance.—The rabbits used in this series were fasted for 12 hr. before the start of the experiment. The drugs, dissolved in isotonic saline, were injected intravenously, followed 40 min. later with 3 Gm. of glucose as a 5% solution. The results are shown in Fig. 1. It is clear from the figure that both tecomine citrate and tecostanine hydrochloride in the dose used, *i.e.*, 20 mg./Kg., reduced the time needed for the blood sugar to return to normal from about 3.5 to 2.5 hr. There was a statistically significant difference between the control values and the values obtained with either drug at 3.5 hr. after the administration of glucose.

Depancreatized Rabbits.—After complete depancreatization, the rabbits developed hyperglycemia in 24 hr. After the hyperglycemia was established and glucosuria produced, tecomine citrate (20 mg./Kg.) and tecostanine hydrochloride (20 mg./Kg.) were injected i.v. Blood samples were collected 3 hr. later, since the alkaloidal salts exhibited maximum hypoglycemic effect at this time when given intravenously (see Table I), and assayed for sugar content. The results obtained are shown in Table II.

A Student *t* test was carried out on the values obtained before and after treatment, and the probability ratios (*P*) are included in the table. From the table it could be seen that the two alkaloids

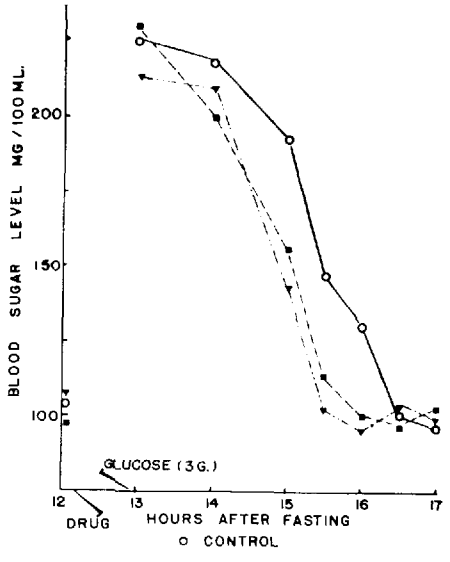

Fig. 1.—Results of glucose tolerance. Key: O, control; ▼, tecomine citrate, 20 mg./Kg.; ■, tecostanine hydrochloride, 20 mg./Kg.

[1] La Roche and Co., Ltd., Basle, Switzerland.

TABLE II.—EFFECT OF INTRAVENOUS ADMINISTRATION OF TECOMINE CITRATE AND TECOSTANINE HYDROCHLORIDE ON THE BLOOD SUGAR OF DEPANCREATIZED RABBITS

Substance Administered	Dose,[a] mg./Kg.	Rt.	Blood Sugar, mg./100 ml. Blood[b] Initial	Maximal[c]	p
Tecomine citrate	20	i.v.	257	238	0.2–0.5
Tecostanine hydrochloride	20	i.v.	301	264	0.1–0.2

[a] Calculated as the free base. [b] Average of four to six experiments. [c] Three hours after the injection of the alkaloidal salts

TABLE III.—EFFECT OF TECOMINE CITRATE AND TECOSTANINE HYDROCHLORIDE ON THE BLOOD SUGAR LEVEL IN ALLOXAN DIABETIC RABBITS

Substance Administered	Rabbit No.	Blood Sugar Level, mg./100 ml. Fasting Before Alloxan	Fasting After Alloxan[a]	Days After Alloxan[d] 16	22	45
Saline (control)	1–4[b]	103.7	275.6	289.7	310.0	
Tecomine citrate, 20 mg.[c]/Kg.	5	105.3	270.4	115.0	124.0	131.3
	6	110.3	320.1	107.3	113.4	123.3
Tecostanine hydrochloride, 20 mg.[c]/Kg.	7	92.7	303.7	100.1	97.2	101.6
	8	99.0	256.4	123.3	139.3	143.1

[a] Before drug treatment was started. [b] Average of four rabbits. [c] Calculated as the free base. [d] Treatment instituted

are not significantly effective in reducing the blood sugar of depancreatized rabbits since the difference between the treated and control is not statistically significant ($P > 0.05$). Doses of 50 mg./Kg. were also given and were unable to produce statistically significant reduction in the blood sugar of the depancreatized animals used.

Alloxan Diabetes.—Alloxan diabetic rabbits with blood sugar above 250 mg. % and exhibiting glucosuria were prepared for this study. The two alkaloidal salts were injected intravenously in isotonic saline. Three hours later, blood samples were collected and assayed for reducing sugar. Only eight animals were used in this study, *viz.*, four controls given saline and four experimental.[2] The results are shown in Table III.

It is clear from Table III that both tecomine citrate and tecostanine hydrochloride in the daily dose of 20 mg./Kg. were effective in reducing the hyperglycemia produced by alloxan to near normal levels. It should be noted that the four controls died on the 23rd, 27th, 29th, and 33rd day after alloxan administration while the experimental animals were living until the 45th day when the experiment was terminated.

DISCUSSION

From the results obtained in this study, it is clear that the salts of tecomine and tecostanine, the two alkaloids isolated from the leaves of *T. stans*, have valuable properties as antidiabetic agents. They are effective both orally and intravenously with a high margin of safety (LD_{50} 300 mg./Kg. in mice) (7), in the doses used in the present study. The two alkaloids present an entirely new nucleus for hypoglycemic activity which provides new possibilities toward the production of hypoglycemic agents. Structure-activity relationships of the new nucleus are presently under study in this laboratory to determine the active site on the two molecules.

From the mechanistic point of view, the two alkaloids seem to need a minimum of active β-cells of the pancreas for action, and in this respect resemble other orally active antidiabetic drugs, *i.e.*, sulfonylureas (10). The two alkaloids were inactive in depancreatized animals. The beneficial effects observed with the two alkaloids in alloxan diabetes are in favor of introducing them for clinical trial. The two alkaloids are without noticeable toxic effects on the rabbits used in the doses applied.

[2] The limited quantities of the alkaloidal salts available prevented the use of a larger number of animals.

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