

INTERACTION OF THE SESQUITERPENE - LACTONES,
AMBROSIN AND DAMSIN, AND GOSSYPOL WITH INSECT
AND SNAIL ACETYLCHOLINESTERASE ACTIVITY.

by

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ABSTRACT

The two sesquiterpene-lactones, ambrosin and damsin which are the major components in the ethanol extract of Damssissa plant of (Ambr-
osia maritima) and gossypol (sesquiterpene) naturally exist in cotton plant were tested against of AChE activity. The enzyme was prepared from house flies Musca domestica as well as Theba pisana (Muler) a terrestrial snails. The effects of the three compounds on the enzyme from both sources were varied. So that, whereas gossypol stimulate the enzyme activity from both sources, the two sesquiterpene-lactones extracted from damssissa, inhibited the enzyme activity from house flies. However, the susceptibility of the enzyme activity from snails towards the two sesquiterpen-lactones were different. So, although ambrosin still inhibit the enzyme activity from this source as well as the house flies, damsin showed a stimulatory effect similar to that of gossypol. On the other hand, although ambrosin inhibited the activity of the enzyme from both sources, kinetic analysis of the inhibition showed different inhibition mechanisms of the both cases. The results were discussed in terms of the chemical structure of the three compounds tested and of the possible variation of the enzyme from the two sources used in the study.

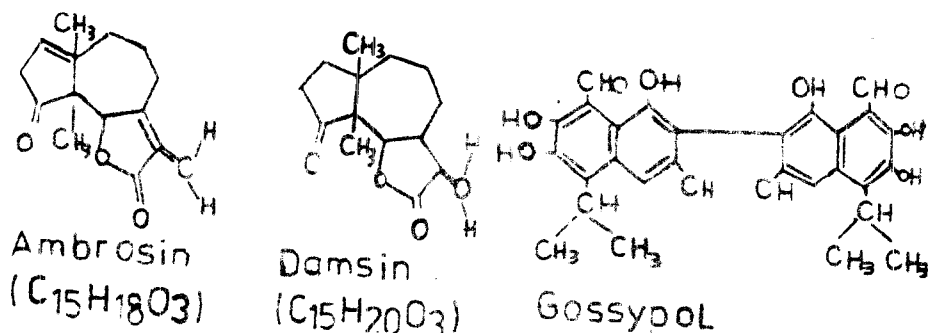
INTRODUCTION

Ambrosia maritima (Damssissa) is an annual herbaceous plant widely distributed through out the Mediterranean region. The dried plant has a characteristically disagreeable odor and very bitter. It has been tested as molluscicide for the control of snails (Sherif et al 1962). Others have used Damssissa as a larvicide and pupicide against Anopheles pharoensis mosquito (El-Sawy et al 1986). These investigators have showed the ability of this plant in terms of biological control of adult mosquitoes. Chemical investigation of this plant led to the identification of two major compounds ambrosin and damsine as sesquiterpene-lactones (Abu-Shady and Sione 1953). In the present study we used these two compounds compared to another known sesquiterpene gossypol , (Stipanovic et al 1977) to test these plant components would have exert their physiological effect through the effect on the cholinergic system of the entire pests or whether they would have another physiological effect. Therefore AChE preparations were obtained from house flies as an insect source and terrestrial snails (Radwan et al, 1991) results indicate that these compounds have a diverse effects on the enzyme from both sources. Nevertheless, evaluation these effects in terms of the physiological action of this plant needs more investigation.

MATERIALS AND METHODS

1- Chemicals

Ambrosin and damsine ($C_{15}H_{18}O_3$, $C_{15}H_{20}O_3$) were a gift from prof. Nabil Abdel Salam, Pharmacognosy Department, Faculty of Pharmacy Alex. Univ. Egypt. Gossypol, technical grade was obtained from Sigma Company. All other chemical reagents were also purchased from Sigma Company.



Chemical structure of Damsin, Ambrosin and gossypol

2- Enzyme sources

- A) Snails, Theba pisana (Muler) were collected from citrus trees in Faculty of Agriculture farm, Abees, Alexandria.
- B) House flies (Musca domestica) were reared in toxicological laboratory, Pesticide Chemistry Alex. Univ.

3- Enzyme preparations

Adult house fly head capsules, mollusca heads and muscular foots were collected as described (Redwan et al 1991) and homogenized for 30 sec in ice cold 100 mM phosphate buffer at pH 7.4. Homogenates were centrifuged at 10,000 xg for 30 min at 4 C°. Debris were discarded and supernatants were saved as source for the enzyme assays.

4 -Assay of acetylcholinesterase (AChE) activity and inhibition:

AChE activity was assayed in the above described supernatants using Acetylthiocholine iodide (ASChI) as substrate. The reaction mixture contained 100mM phosphate buffer PH 7.4, .5 mM ASChI and .5 mM DTNB [5,5'-dithio-bis-(2-nitrobenzoic acid)]. The reaction was started by adding AChE (100 ug) for 1min incubation time

Table (1) Effect of Sesquiterpene Lactone (Ambrosin and Damsin) on AChE isolated from house flies and snails.

Conc. [μM]	<u>Enzyme Activity as % of Control</u>			
	house flies		snails	
	A*	D*	A*	D*
0.0	100.0	100.0	100	100
5	69.1	71.1	70	101
10	57.1	69.0	64	108
15	41.9	58.2	60	138
20	38.9	41.0	49	138
25	36.3	41.0	43	138
30	33.0	41.0	39	137

A*: Ambrosin
D*: Damsin

Table (2) Effect of gossypol on AChE isolated from house flies and snails

Conc. [μM]	<u>Enzyme Activity as % of Control</u>	
	house flies	snails
0.0	100	100.0
5	115	148.0
10	119	140.0
15	122	136.0
20	127	135.0
25	124	121.0
30	124	110.0

Control activity was 7.16 O.D at 412nm/mg protein/min and 6 O.D at 412nm/mg protein/min for house fly and snail.

and will be produce thiocholine iodide which is determined colorimetrically at 412 nm (Ellman et al 1961).Assessement of the enzyme inhibition by the tested compounds ambrosine, damsine and gossypol were conducted by adding different concentrations of these compounds to the enzymatic reaction mixtures contained same concentration of the supernatant protein (AChE) and different concentrations of the substrate ranged from 50 - 500 μ M with and without fixed concentration of ambrosin(10 μ M) The type of inhibition was determined using lineweaver-Burk method (1934).

RESULTS AND DISCUSSION

The data in table(1)shows that the two sesquiterpene - lactones , compounds ambrosin and damsine , can inhibit the AChE activity of the house fly preparations. However the effect of these two compounds on similar preparations from snails were varied. So, whereas, ambrosin still inhibit such activity, damsine failed to inhibit the enzyme activity in this case, and higher concentrations (10 - 30 μ M) showed stimulation.

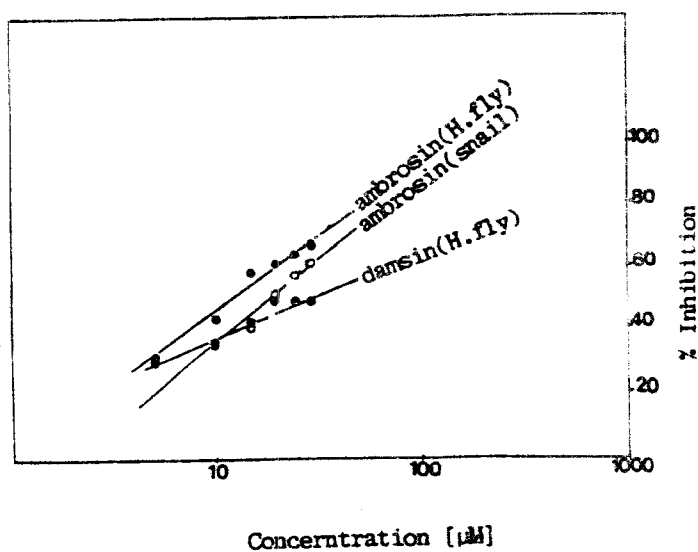
El-Sawy et al(1986)found that the dry damssissa plant preparations have lethal effects on belharzia snails as well as larvae and pupae of Anopheles pharoensis in the laboratory.

Analysis of the ethanol extract of these preparations showed that the two compounds used in the present study, ambrosin and damsine are major components of the damssissa extract.

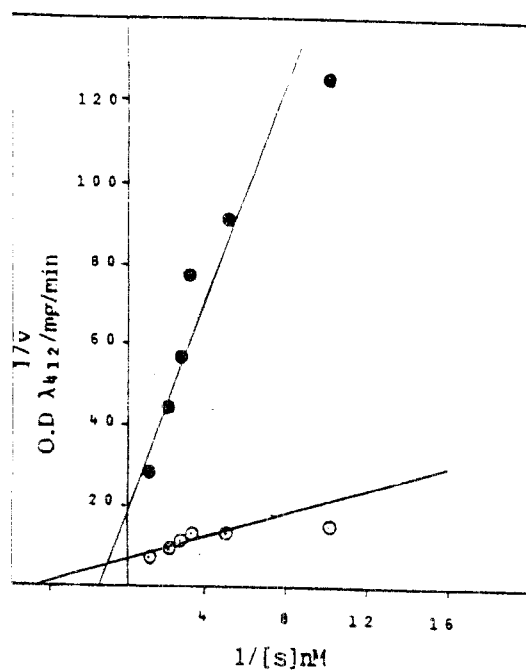
Gossypol, another sesquiterpene which exist in cotton plants (Sherby, 1979) showed similar effects on the enzyme activity of both preparations to that of damsine on the preparation from snails. Concentrations of gossypol between (5 to 30 μ M) showed stimulation of the enzyme activity. The same range of concentration from ambrosin was inhibitory to the enzyme activity from both source. Damsine effect was similar to ambrosin in the case of house flies and to

Table (3): Parameters of inhibitory type of ambrosin and IC₅₀ of other chemicals on house fly and snail acetylcholinesterase(AChE).

Parameter	Value	
	house fly AChE	snail AChE
V _{max} (O.D ₄₁₂ /mg/min)	0.167	0.33
V _{max app} (O.D ₄₁₂ /mg/min)	0.05	0.22
K _m (μM ⁻¹)	0.277	0.059
K _{m app} (μM ⁻¹)	0.714	0.04
K _i (μM)	4.27	20
IC ₅₀ values		
ambrosin	12 μM	19 μM
damsin	22 μM	-
gossypol	-	-



Fig(1): Relation between log concentration of sequiterpenes and % inhibition on house fly and snail AChE.



Fig(2): Double reciprocal plots of $1/v$ versus $1/[S]$ for house fly AChE in presence of (●) and absence of 10 μM ambrosin (○).

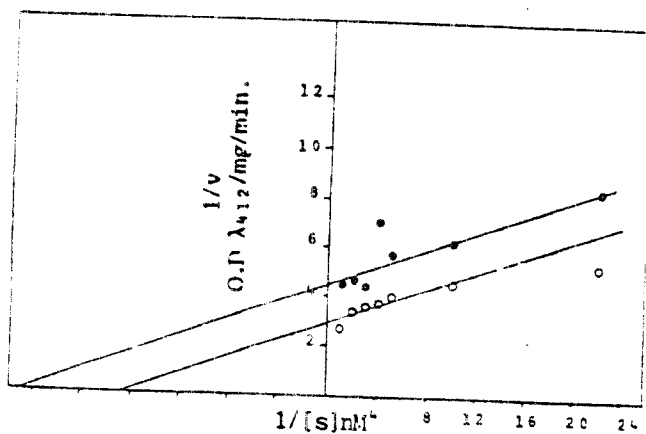


Fig (3): Double reciprocal plots of $1/v$ versus $1/[S]$ for snails AChE in presence of (●) and absence of 10 μM ambrosin (○).

gossypol in the case of snail preparations. The difference in chemical structure between gossypol and the two sesquiterpene-lactones is apparent and can be thought of as bases for their differential effects on the enzyme activity. However, the differences between the two sesquiterpene-lactones are not that much. chemical studies of these two compounds by Abo-Shady and Soine 1953, summarized differences in the positions of ambrosin structure of a conjugated carbonyl group. this difference in structure can be seen in their different effect on the enzyme from both sources, which may predict a role for such structure in the enzyme inhibition. Figure (1) shows IC_{50} values of ambrosin and dampsin on the two enzyme. Table (3) and figures (2, 3) indicate that the type of the enzyme inhibition from both sources by a fixed concentration of ambrosin, (10 μM) in the presence of varied concentrations of substrate noncompetitive in the case of house fly preparations and uncompetitive with snail enzyme. This difference in the type of inhibition may be due, either to endogenous factors in the preparations, or to an intrinsic differences in the enzyme itself from both sources. In essence the present data shed some light on the possible biochemical bases of biocidal effects of damssissa plant on insects and snails.

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تداخل الدامسين والامبروسين من مرهبات
السيستوفيرين-لاختون والجوسيبول مع
انزيم الاسيتيل المعزول من القواقع
والذباب المنزلي
=====

نظرا لما للمرهبات الطبيعية المستخلصة
من النباتات دور هام في مكافحة الافات
في هذه الونة لتجنب المزيد من تلوث
البيئة وبالتالي التأثير على مه
الصحة فان هذه الدراسة استهدفت
استخدام تلك المستخلصات على بعض النظم
الحيوية لبعض الافات الغاية في الخطورة
سواء على النباتات أو الصحة العامة.
فاشارت الدراسة بأن استخدام
السيستوفيرين-لاختون والتي منها
الدامسين والامبروسين بمدى من
التركيزات من ٥-٣٠ ميكرومولر ادت الى
انخفاض في النشاط الانزيمي الى ٢٥% لكلا
المصدرين ما عدا الدامسين الذي ادى
الى زيادة نشاط الانزيم المعزول من
القواقع.

وعلى الصعيد الاخر فان مرهبات الجوسيبول
كان له تأثير تنشيطي على كلا الانزيمين
وهو من مجموعة السيستوفيرين.
كما بينت دراسة هرهية التفاعل الانزيمي
ان التنشيط من النوع اللاتنافسي للانزيم
من كلا المصدرين.