Isolation and Partial Characterization of the Toxic Substance from EREMOCARPUS SETIGERUS BENTH

Shuliang Lee

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ISOLATION AND PARTIAL CHARACTERIZATION OF THE TOXIC SUBSTANCE FROM EREMOCARPUS SETIGERUS BENTH

by

Shuliang Lee

A Thesis in Partial Fulfillment of the Requirements for the Degree Master of Science in the Field of Chemistry

May 1968
Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate, in scope and quality, as a thesis for the degree of Master Science.

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ACKNOWLEDGMENTS

The author wishes to express grateful appreciation for the kind assistance and patient guidance of Dr. William D. Leech and Dr. Laurence W. Botimer in this research.

The author also expresses his sincere thanks to Dr. George J. Nelson, Dr. Earl W. Lathrop, Mr. H. Raymond Shelden, Mr. Dennis H. Dale, and Mrs. Pauline M. Baldwin for their assistance in this work.
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INTRODUCTION

Numerous plants were used as fish poisons by California Indians. Among these, the two most highly esteemed were Chlorogalum pomeridianum (soap root), a member of the lily family, and Eremocarpus setigerus Benth (turkey mullein), a member of the spurge family.\(^1\) The active component of the former is saponin, which has a hemolytic effect on erythrocytes and causes the erythrocyte cell membrane to burst. Upon hydrolysis, saponin yields sugars and a steroid sapogenin, which is a potential precursor for sex and cortical hormones.\(^2,3,4\) On the other hand, the active principle of turkey mullein has not yet been elucidated. Naito and Noller\(^5\) attempted to solve this problem by solvent extraction and column chromatography without too much success. Therefore we have tried to obtain a pure sample of this substance using more selective processes and by means of microanalytical methods using colored spot tests to determine the nature of the substituent on the molecule.
REVIEW OF LITERATURE

**Eremocarpus setigerus Benth**, a member of the spurge family, is an annual, heavy-scented, short gray weed which is native to the Pacific coastal states from Southern California to Washington. It also appears on the San Clemente and Santa Catalina islands which are located in the Pacific Ocean southwest of Long Beach and northwest of San Diego, California. This annual weed grows abundantly, from July to November, on dry undisturbed soil, in stubble fields, or waste-places at an elevation of 1,000 to 2,500 ft above sea level.

The plant is very conspicuous because of its mat-like, circular shape. It is arranged in flat, leafy rosettes with white, bristly hairs, which profusely cover the whole plant. This is a distinguishing characteristic. The bristle is a part of the compound hair, which radiates outward from the leaf in all directions. Both the flowers and the fruit are inconspicuous. The bean-like seeds appear shiny, and are about a sixth of an inch long. They are produced in great abundance. These seeds constitute one of the favorite foods of the mourning doves and turkeys. For this reason, and on account of the woolly, mullein-like appearance of the leaf, the plant has often been called "dove weed" or "turkey mullein."

During the summer season, when the river was running low, the California Indians would gather this heavy-scented weed into bundles and crush them up between the rocks. With workers stationed along the stream for a couple of miles, bundles of the crushed plant were thrown into the water and were submerged in the stream by weights.
After a short time, all the fish in the vicinity of the plant were stupefied by the poison and floated on the surface of the water. Then the Indians captured them either by hand or by the use of a shallow, coarse-meshed basket.\(^8\)

Although the actual toxic principle of the plant was not known, the Indians speculated that the killing action could be attributed to the stellate hair which attached to the gills and the eyes of the fish. However, this belief can be easily discredited by carefully examining the presence of the stellate hair in the gills and the eyes of the fish caught by means of this plant.\(^9\)

Although the plant is toxic to fish, there is no report of ill effects on the human body as a consequence of eating the fish thus poisoned. Furthermore, the Indians used this plant for medicinal purposes. They applied the bruised leaves to the chest as a counterirritant for internal pain. A weak solution of the plant was used as a bath in typhoid disease and also was taken internally as a cure for chills and fever.\(^10\)

It was first reported by Hall and Yates\(^11\) that sheep and Belgian hare had been killed by feeding on the plant. They attributed the death to the action of the stellate hair on the alimentary tract of the animal. Later Kingsburg\(^12\) reported that cattle feeding on *Eremocarpus setigerus* Benth developed symptoms of stiff gait, arched back and diarrhea, and there was an abrupt decrease in milk production in dairy animals.

Naito and Noller\(^5\) have been the first in recent years to search for the active component of this plant. They applied solvent extraction and column chromatography techniques to the problem, but failed
to obtain a pure sample of the toxic substance. They pointed out that
the toxic substance is free of halogen, nitrogen, and sulfur, and is
not a water soluble saponin. Since highly toxic croton oil was found
in *Croton tiglium*, which also is a member of the spurge family, they
tried to correlate this active component to the croton oil and resin
by means of chemical reactions. However, the results were all nega-
tive.
EXPERIMENTAL

The plants used were gathered along the roadside of U. S. Highway 38, which, with an elevation of 2,000 feet above sea level, leads to the mountain region of San Bernardino County. A total of 333.5 lb of plants were collected during the period from the middle of September to the early part of November. After being removed from the field, each batch of approximately 50 lb of fresh plants was disposed of immediately, within three days, to avoid excessive loss of volatile substances.

To show the erroneous character of the belief of California Indians that the killing action on fish may be attributed to the plant's hair, the following preliminary toxicity tests on goldfish were made in accordance with the method of Gersdoff.13

Into each of four 6-inch wide-mouth jars, a liter of tap water, previously regulated at 23° C (our room temperature), was introduced. To assure that no hypoxia condition should occur in the environment, sufficient air supply from the air line was permitted to pass through a carbon filter, into a fourway glass manifold, and bubbled into the content of the jars through capillary delivery tubes. After the water had come to equilibrium with its surroundings a 2-1/2-inch healthy Comet goldfish was placed into each of the jars for at least 12 hr.

We wish to thank Dr. Earl W. Lathrop for identification of the plants collected.

A healthy goldfish has to satisfy the following criteria: active and no appearance of unusual swimming behavior; luster in color; erected fins; and no excretions remaining attached to the vent in long thin strings.
prior to their new environment, and also permitted us to observe any malady which might affect the fish during this transition.

Then, into jar A, 20 g of crushed fresh plant were introduced; into jar B, 10 drops of the concentrated yellowish-brown ethereal extract from the steam distillation of 4 kg fresh plant leaves were added, and 10 drops of ethyl ether were introduced into jars C and D. The fish in jar B died within 75 min, and fish in jar A expired within 360 min. Both fish showed the following stages of distress: (1) drooping of fins; (2) retardation of movement; (3) loss of body equilibrium; (4) death. As for subjects C and D above, symptoms were not observed. This supports the view that the killing action on fish may be attributed to some chemical substances within the plant rather than to the plant's hair alone.

Upon evaluation of the preliminary toxicity tests, we assumed that the pungently odorous substances from the plant leaves account for the results. Basing our procedure on this premise, we adopted a steam distillation process rather than the solvent extraction used by Naito. In order to speed up the process, four steam distillations were run simultaneously. Each distillation required an hour. The steam-volatile yellow oils were carried over to the receiving flask. The distillate was then saturated with sodium chloride to salt out the oils. Next we extracted the distillate successively with 10 ml, 5 ml, and 5 ml of ethyl ether. The slightly yellowish ethereal phases were combined and dried over magnesium sulfate. This process was repeated over a period of four weeks until a total of 333.5 lb fresh plant had been processed. The ether was removed from the combined

It is interesting to mention at this point that the author
extracts leaving 30 ml of oily residue. The oils were now vacuum distilled in order to separate them without decomposition. Several fractions were obtained with a 30 cm Vigreaux fractionating column at a pressure of 0.65 mm of mercury, as follows:

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Description</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>pale yellow liquid</td>
<td>56° - 60°</td>
</tr>
<tr>
<td>II</td>
<td>pale yellow liquid</td>
<td>60° - 74°</td>
</tr>
<tr>
<td>III</td>
<td>pale yellow liquid</td>
<td>74° - 83°</td>
</tr>
<tr>
<td>IV</td>
<td>yellowish liquid</td>
<td>83° - 101°</td>
</tr>
<tr>
<td>V</td>
<td>yellow-orange liquid</td>
<td>101° - 120°</td>
</tr>
<tr>
<td>VI</td>
<td>brownish residue</td>
<td></td>
</tr>
</tbody>
</table>

In order to eliminate the nontoxic fractions, another toxicity test on goldfish was made. Eight healthy 2-1/2 inch Comet goldfish were used: two as controls and the others as experimental subjects for the various oil fractions. The testing conditions were identical with those of the preliminary toxicity test. Two micro drops of each fraction were administered separately to the individual subjects. The fish tested with fractions IV and V died within 147 min and 152 min respectively; the others survived. This indicates that the active substances were in the two high boiling fractions.

Elementary analysis\(^{14}\) of fractions IV and V showed that these fractions contained no halogen, sulfur, and nitrogen.

The solubility tests\(^{15}\) of these fractions revealed that the active substances were probably neutral compounds such as alcohols, experienced having the skin peel off his fingers by handling the plant with bare hands for two weeks. Later surgical gloves were worn.
aldehydes, ketones, esters or unsaturated compounds. They were soluble in ethanol (95%), ethyl ether, and cold concentrated sulfuric acid with brownish-red coloration. They were insoluble in water, dilute acid or base.

The next attempt was to further isolate and purify the active substances from these fractions. In this work, thin layer chromatography was the primary tool for further separation of the fractions, and gas chromatography was used to check the purity of each separated portion.

The chromatographic plates or chromatographic strips were prepared by mixing 30 g of Silica Gel G with 65 ml of distilled water. The slurry, free of air bubbles, was transferred into a Stahl thin layer spreader and was spread to a thickness of 250 μm on 4 glass plates (20 x 20 cm) or 16 glass strips (5 x 20 cm). The coated plates and strips were allowed to air dry for twenty minutes, then transferred to a drying storage rack. The rack was laid in an oven so that the plates and strips were vertical, baked them for 2 hr at a temperature of 110° C. After this period of activation, we removed and stored them in a desiccating cabinet for later use.

To ensure a good separation of these fractions, two solvent systems were used: (1) benzene, and (2) benzene-absolute ethanol (95:5 v/v). Most nonpolar substances were developed by the first system, and polar substances were further separated by the latter system.

For the qualitative purpose, chromatostrips (5 x 20 cm) were adopted. A micro-drop of each active fraction was spotted individually,
2 cm from the bottom edge of the strip. After the material was absorbed on the thin layer, the strip was developed by the ascending technic in a chromatographic chamber previously saturated with benzene. When the solvent ascended to a distance of 15 cm, the development was terminated.

To give visual identification of the compounds, several detection sprays were used, i.e., antimony trichloride in alcohol-free\(^d\) chloroform (1:3 w/w); antimony pentachloride in CCl\(_4\) (1:4 v/v); fluorescein/bromine; 1% vanillin in 9% H\(_2\)SO\(_4\); 2,4-dinitrophenylhydrazine in 2N HCl; and chlorosulfonic acid in acetic acid (1:2 v/v). The Rf values and color appearance were listed in Table I. (Also refer to Plates I and II.)

In the case of quantitative separation, chromatographic plates, and microstrips of sample were used instead. After the development with benzene, during which a plate with parallel lines of each fraction was formed, we masked all but one edge (2 cm) and sprayed this edge with antimony trichloride in order to give a color by which the various fractions could be located. The remaining uncolored section of the plate was divided up for removal of the Silica Gel G with adsorbed product. These fractions are designated as portions. Each portion was removed from the Silica Gel G by extraction with ether, and the ether was removed in a water bath of 45° C to give a sample of the pure substance.

\(^d\)The alcohol-free chloroform was obtained by eluted commercial Baker analytical grade chloroform through a pH 9 alumina column. The pH 9 alumina was obtained by washing the neutral alumina with a pH 9 buffer solution.
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Portion</th>
<th>Spot</th>
<th>Rf</th>
<th>2,4 Dinitrophenylhydrazine</th>
<th>SbCl₃</th>
<th>Sprayed with Chlorosulfonic acid</th>
<th>Vanillin in H₂SO₄</th>
<th>Fluorescein Br₂</th>
<th>SbCl₅</th>
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<tbody>
<tr>
<td>IV</td>
<td>1</td>
<td>1</td>
<td>0.0</td>
<td>y</td>
<td>rsa</td>
<td>rsa</td>
<td>ORBr</td>
<td>y</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>0.14</td>
<td>lrrsa</td>
<td>lrrsa</td>
<td>lrrsa</td>
<td>drv</td>
<td>y</td>
<td>dv</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.26</td>
<td>v</td>
<td>rsa</td>
<td>v</td>
<td>rsa</td>
<td>lr</td>
<td>y</td>
<td>dv</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>1</td>
<td>0.0</td>
<td>rsa</td>
<td>rsa</td>
<td>rsa</td>
<td>ORBr</td>
<td>y</td>
<td>dv</td>
</tr>
<tr>
<td></td>
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<td>2</td>
<td>0.16</td>
<td>lrrsa</td>
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<td>lrrsa</td>
<td>drv</td>
<td>y</td>
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<td>v</td>
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<td>v</td>
<td>rsa</td>
<td>lr</td>
<td>y</td>
<td>v</td>
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<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>0.67</td>
<td>rsa</td>
<td>rsa</td>
<td>rsa</td>
<td>rsav</td>
<td>yOR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>0.74</td>
<td>fy</td>
<td>fv</td>
<td>fv</td>
<td></td>
<td>yOR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>0.90</td>
<td>OR</td>
<td>fv</td>
<td>v</td>
<td>dv</td>
<td>y</td>
<td>dv</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
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<td>frsa</td>
<td>rsa</td>
<td>rsa</td>
<td>v</td>
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<td></td>
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<td></td>
<td>4</td>
<td>5</td>
<td>0.67</td>
<td>rsa</td>
<td>rsa</td>
<td>rsa</td>
<td>rsav</td>
<td>yOR</td>
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<td>5</td>
<td>6</td>
<td>0.76</td>
<td>fy</td>
<td>fv</td>
<td>fv</td>
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<td></td>
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<tr>
<td></td>
<td>6</td>
<td>7</td>
<td>0.90</td>
<td>fv</td>
<td></td>
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</table>

**Color:**  
d = dark; 1 = brilliant; f = pale; bl = blue; Br = brown; y = yellow; r = red, OR = orange; v = violet; rsa = pink; g = green; n = no.

*Benzene solvent.
Plate I eluted with benzene. Plate II eluted with benzene-absolute ethanol(95:5v/v). Silica gel G(250μm of thickness). Length of run: 14-15cm. Samples: a-fraction IV, b-fraction V, c-portion 2 of fraction IV, d-portion 2 of fraction V. Spray reagents:
1) 2,4 dinitrophenyl hydrazine 2) SbCl₃ 3) Chlorosulfonic acid
4) vanillin/H₂SO₄ 5) fluorescein/Br₂ 6) SbCl₅. Photo by Dennis H. Dale.
Another toxicity test showed that portion 2 from fractions IV and V are equally toxic to goldfish.

In a slight modification of separation, gas chromatography was used. A 0.5 μl sample of portion 2 was injected into an 8-foot x 1/4-inch copper column containing 5% (w/w) carbowax 2M coated on 60-80-mesh neutroport T for temperature programming from 70°C - 120°C. Two major components besides the ether and some contaminants were observed. But their retention times were relatively close (see Fig. 1). This indicated that these two substances are quite similar in polarity.

Since it was quite difficult to separate them by gas chromatography, we returned to thin-layer chromatography by using benzene-absolute ethanol (95:5 v/v), a more polar solvent system, to resolve them. Finally, spots 2 and 3 (see Table I) were distinctly separated with a Rf value of 0.57 and 0.68 respectively.

The UV and IR spectra of these two substances were recorded by a Hitachi Perkin-Elmer 139 UV-VIS spectrophotometer and a Beckman IR-4 as indicated on Figs. 2, 3, 4 and 5. The Beckman IR-4 was standardized with polystyrene before use. In the preparation of IR spectra, we found that coating the substance to be examined directly onto a previously-formed KBr pellet was simple and satisfactory.

A final toxicity test showed that spot 3 has no effect on the goldfish, but with concentration of two parts per million of spot 2, the mean survival time of the goldfish was 223 min.

Further characterization of the active substance was done by means of spot tests\textsuperscript{17} for functional groups. Each functional group test done on the active compound was also performed on a known compound for comparison purposes. The results are listed on Table II.
FIG. 1 - GAS CHROMATOGRAPHY OF PORTIONS 2 FROM FRACTIONS IV AND V

Fraction IV

Retention time

1 2 3 4 5 6

Fraction V

Retention time

0 1 2 3 4 5 6

a = ethyl ether. b and c = principal materials of portion 2 from fractions IV and V. d, e, and f = contaminants. Sample injected: 0.5 μl. Temperature programming: 70° - 120° C. Rate: 40° C/min.
FIG. 2 - ULTRAVIOLET SPECTRUM OF SPOT 2 (TOXIC SUBSTANCE)

Wavelength in m$\mu$

Absorbance (log I/I$_0$)

204 m$\mu$ sample: 3.5 mg/100 ml of 95% ethanol
FIG. 3 - ULTRAVIOLET SPECTRUM OF SPOT 3

sample: 1.2 mg/100 ml of 95% ethanol

Wavelength in m\(\mu\)

Absorbance (log \(I_0/I\))

203.5 m\(\mu\)
FIG. 4b - INFRARED SPECTRUM OF SPOT 2 (TOXIC SUBSTANCE)

- **Sample:** Spot 2 coated on KBr pellet
- **Reference:** Air
- **Speed:** 0.5 μ/min.
- **Gain:** 3.0%
- **Period:** 8
- **Slit Schedule:** Std.
- **Graph Scale:** 66.5% of the origin
FIG. 5a - INFRARED SPECTRUM OF SPOT 3
FIG. 5b - INFRARED SPECTRUM OF SPOT 3

- **Sample**: spot 3 coated on KBr
- **Reference**: air
- **Speed**: 0.5 μ/min.
- **Gain**: 3.0%
- **Period**: 8 slit sched: Std.
- **Graph Scale**: 66.5% of the origin
### TABLE II
SOME COLOR REACTIONS OF THE TOXIC SUBSTANCE COMPARED WITH KNOWN COMPOUNDS

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Ref. No.</th>
<th>Toxic Substance</th>
<th>Anisaldehyde</th>
<th>Crotonaldehyde</th>
<th>Cyclohexane</th>
<th>Salicylic Acid</th>
<th>Ethyl Acetate</th>
<th>Cholesterol</th>
<th>Eucalyptus</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥C=CH=C=</td>
<td>21</td>
<td>rrsa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>22a</td>
<td>nvbl</td>
<td>v</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nvbl</td>
</tr>
<tr>
<td>-C-H</td>
<td>22b</td>
<td>yg</td>
<td>dv</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>yg</td>
</tr>
<tr>
<td>≥C=O</td>
<td>24</td>
<td>v</td>
<td>dv</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>O</td>
<td>25</td>
<td>v</td>
<td></td>
<td>v</td>
<td>y</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>-C=OR</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Triterpenoid</td>
<td>29a</td>
<td>lrrsa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fy</td>
<td>yOR</td>
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<td>29b</td>
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<td></td>
<td></td>
<td></td>
<td>yOR</td>
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</tr>
<tr>
<td>Pentacyclic triterpenoid</td>
<td>31</td>
<td>fy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fy</td>
</tr>
</tbody>
</table>

Color symbols: refer to Table I.
22a = Schiff reagent; 22b = 2,7-diaminofluorene; 29a = chlorosulfonic acid; 29b - vanillin/HCl.
DISCUSSION OF RESULTS

Since the technique used by Naito was not satisfactory, we adopted steam distillation and thin layer chromatography technique to resolve the problem. This technique enabled us to isolate the toxic substance in a pure state. Our elementary tests, which showed that the toxic substance was free of halogen, nitrogen, and sulfur, correspond to those reported by Naito. The toxic substance chromatographed on Silica Gel G had Rf values of 0.15 in the benzene system and 0.57 in the benzene-absolute ethanol (95:5 v/v) system. This, accompanied by the solubility test, indicates that it is a neutral compound such as alcohol, aldehyde, ketone, ester or unsaturated hydrocarbon or a combination of these.

A maximum absorption at 204 m\(\mu\) in the ultraviolet region and a yellow coloration with fluorescein-bromine reagent indicate that the substance has an isolated double bond. Furthermore, a positive red pink coloration with phloroglucinol-hydrochloric acid indicates the double bond is of a trisubstituted nature (\(\equiv C-CH=C\approx\)), for the propenyl (\(-CH=CH-CH_3\)) or the vinyl (\(-CH=CH_2\)) group would not give the color reaction.

The absence of nitrogen in the element test and the presence of two absorption bands at 2.93\(\mu\) (3420 cm\(^{-1}\)) and 8.7\(\mu\) (1150 cm\(^{-1}\)) in the infrared region indicate the existence of a saturated tertiary or a highly symmetrical secondary hydroxyl group.

Negative tests with Schiff reagent and 2,7-diaminofluorene shows the absence of an aldehyde group but a blue color with bisulfite-potassium iodide-starch test indicates the presence of a ketone.
group. Furthermore, a fragrant odor from the toxic substance as well as a positive hydroxamic acid test\textsuperscript{24} with ferric chloride implies the presence of an ester function.

A positive red coloration with ferric chloride\textsuperscript{25} in chloroform solution indicates the presence of an enol group. This enol group
\[ \text{QH} (-\overset{\text{=}}{\overset{\text{CH-}}{\text{O}}} \text{H}) \]
could not be attributed to the conjugation of the double bond and the hydroxyl group. For, if it were, the hydroxyl group could not be a saturated tertiary or of a highly symmetrical secondary nature. However, this enol form could be contributed by the ketonic function. In addition, a red pink instead of a yellow orange coloration with 2,4-dinitrophenylhydrazine solution\textsuperscript{26} indicates that the parent carbonyl compound has a double bond conjugated with the carbonyl group. This seems to contradict the concept of an isolated double bond shown previously in the ultraviolet absorption. But if this ketone group were conjugated with the ester function at a $\beta$-carbon position, an
\[ \text{QH} \overset{\text{=}}{\overset{\text{CH-}}{\text{C-OR}}} \]
enol form of \((-\overset{\text{=}}{\overset{\text{CH-}}{\text{O}}} \text{H})\) could occur to some extent. This phenomenon would then constitute a positive test for the enol function and for the red pink coloration with 2,4-dinitrophenylhydrazine solution. This concept is further supported by the existence of two peaks\textsuperscript{27} at 5.72$\mu$ (1740 cm\textsuperscript{-1}) and 5.82$\mu$ (1715 cm\textsuperscript{-1}) in the infrared region, which resemble the carbonyl absorptions of ethyl acetoacetate.

Furthermore, the active compound responded to the general terpene-terpenoid reagents.\textsuperscript{28} It especially gave red or reddish-violet coloration with acetic anhydride-sulfuric acid, chlorosulfonic acid, and vanillin in hydrochloric acid. Gerlach\textsuperscript{29} has stated that the last
two color reactions are characteristic of triterpenoid compounds. Belić³⁰ has also reported that triterpenoid glycosides develop red coloration with acetic anhydride-sulfuric acid (Liebermann-Burchard reagent). However, the fact that no violet color developed with 2,6-di-tert-butyl-4-methylphenol³¹ led us to believe it is not of the ursane, oleanane, or Lupane pentacyclic triterpenoid series.
CONCLUSION

According to the above experimental data, we have tentatively concluded that the yellow sticky toxic substance belongs to the triterpenoid series with an isolated double bond, a saturated tertiary or a highly symmetrical secondary hydroxyl group, and a β-ketoester group as part of its structure. A more definite assignment of structure might be made on the basis of carbon-hydrogen analysis, nuclear magnetic resonance, and mass spectroscopic studies.
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26. Ibid., 126 (1965).


ISOLATION AND PARTIAL CHARACTERIZATION OF THE TOXIC
SUBSTANCE FROM EREMOCARPUS SETIGERUS BENTH

by
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ABSTRACT

The work on the active substance of Eremocarpus setigerus Benth has been previously performed by Naito and Noller. However, the investigation was not complete. Therefore we isolated and tested this substance by steam distillation and thin layer chromatography technique. A yellow sticky toxic substance was isolated with Rf values of 0.15 in benzene system and 0.57 in benzene‐absolute ethanol (95:5 v/v) system.

The toxic substance which is free of halogen, nitrogen, and sulfur, is soluble in ethyl ether, ethanol, and concentrated sulfuric acid. It has a maximum absorption in the ultraviolet region that indicates the presence of unsaturation. Its infrared spectrum indicates the presence of a saturated tertiary or a highly symmetrical secondary hydroxyl group and carbonyl group. The functional group tests also indicate the presence of ketone, ester, enol and isolated double bond groups. We believe that the enol form is attributed to the conjugation of the ketone and the ester groups at a β-carbon position.

Furthermore, the toxic substance gave a positive terpene-terpenoid test. This leads us to believe that the toxic substance might belong to the triterpenoid series.