Constituents of Ipomoea pes-caprae L. R. Br.

by

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OBJECTIVES

The work described in this thesis was directed towards the isolation and structure elucidation of constituents of the plant, *Ipomoea pes-caprae* which might have significant anti-cancer activity. The primary goal of the research was to isolate and characterize an active anti-cancer compound, and the secondary goal was to isolate and characterize other new or unusual plant constituents.
INTRODUCTION

In the fight against cancer the primary concern has been with the treatment of patients. The attempts of scientists have been to understand the causes as well as to find cures for cancer. This is not an easy task because cancer, unlike many other diseases, is not a single disease, is not a localized disease, and is not a disease in which the causative agent can be found.¹ As of present the causes of cancer are mostly speculative and the cures have met with limited success.

Of the causes of cancer some speculation has been that environmental factors are the major source.² These environmental factors in many instances are considered self-induced such as additives in food and drinks, drugs, radiation and working conditions.

Efforts to treat patients have largely centered around surgery, radiotherapy and chemotherapy alone or in combinations.³ These methods have proved very effective in the early stages of cancer to eliminate tumor cells. In the later stages of cancer, treatment will depend on further advances in molecular biology, biochemistry, immunology, pathology and clinical science.

Chemotherapy is considered at present the best approach to fighting cancer in certain cases not only because of its immediate therapeutical value, but also because it provides valuable information necessary for advances in the systematic treatment for the later stages of cancer. It is the only method for the treatment of disseminated cancers such as leukemia, and is usually a valuable adjunct to surgical or radiotherapeutic procedures. At present about 45 anti-cancer drugs are used medically and another 40 are in preclinical testing.⁴
In the search for new active anti-neoplastic agents, new active compounds may be sought either by the synthesis of new compounds with presumed activity or by the isolation of compounds from natural sources which are known to be active. Although the first approach is undoubtedly the simpler one, the types of compounds it yields are usually variations on a well-known theme. The natural products approach, however, is capable of yielding quite novel active compounds and thus providing a whole new dimension to the chemotherapy field. The active plants or other natural materials are generally found by a random screening process, whereby several thousand plant species are collected and their extracts tested for activity. The plants yielding active extracts are then collected in larger quantity and subjected to detailed chemical examination.

One plant discovered through this procedure was *Ipomoea pes-caprae*. This plant, which belongs to the morning-glory family, has a history of folk-medicine usage for various ailments. Thus it is known to have been used in New South Wales for rheumatism, dropsy, sores and ulcers. There have been reports of the medical use of *Ipomoea pes-caprae* in *Indian Materia Medica* for the treatment of gout, gonorrhea and old age. In addition the seeds of morning-glory plants are known to contain alkaloids of the ergot family.

The water extract of *Ipomoea pes-caprae* was tested by the National Cancer Institute and found to show good anti-cancer activity for Walker carcinosarcoma 256 (subcutaneous). This test was based on the inhibition of cancer growth in a rat as measured by the growth of the solid tumor
in the treatment animal versus that of a control animal. The testing for anti-cancer activity on P388 lymphocytic leukemia cancer in mice showed no activity. A phytochemical study of Ipomoea pes-caprae for locating anti-cancer activity was considered appropriate at this time. With the aid of the U.S. Department of Agriculture a shipment of dried stems, roots and leaves was collected in Panama and was sent to Virginia Polytechnic Institute and State University for further investigation.

The Morning-Glory family (Convolvulaceae) of which Ipomoea pes-caprae is a member consists of approximately 1600 species in 51 genera. This family is comprised chiefly of tropical and sub-tropical climbers, however, species such as Ipomoea purpurea, the common morning-glory, can be found in the north temperate zone. Of the 51 genera Ipomoea is the largest genus in the family and is known to have some 400 species. Among these species is Ipomoea batatas, the sweet potato, which has long been known as a food source.

Ipomoea pes-caprae, a perennial plant as all members of this family are, is a twining-trailing vine which grows on tropical beaches throughout the world. It consists of tough woody roots which grow from a crown omni-directional up to many feet in length. The roots when young appear purple or flesh color, but become woody appearing as the plant matures. The leaves are described as simple, thick and alternating and are 6-10 cm long. They are round or cordate at the base and rolled up longitudinally as signified by the latter word "convolutus." The flower has pink colored petals 4-5 cm long and sepals that are oval and obtuse shaped. The seeds are formed in a 1.5 cm capsule which opens to let the seeds be spread by the oceans and seas. Ipomoea pes-caprae is
Ipomoea pes-caprae

Photo by Dr. Julia F. Morton
University of Miami
stated by Guppy to monopolize the thirtieth parallels of north and south longitude. In the United States *Ipomoea pes-caprae* can be found on sandy beaches from South Carolina to Texas.

**Taxonomy**

**Plant Kingdom**

**Angiosperms**

Division: Anthophyta

Class : Dicotyledonae

Order : Solanales

Family : Convolvulaceae

Genus : Ipomoea

Species : Pes-caprae

*Ipomoea*; from Gr. "ips," bindweed and "homoios," referring to the twining habit.

*Pes-caprae*; from Lt. "pes," foot and "caprae," goat, foot of a goat, referring to the appearance of the roots.

**Bioassay Testing Systems**

The bioassay testing systems sponsored by the National Cancer Institute and carried out under contract by biomedical laboratories serve two purposes in natural products anti-cancer research. The first purpose is to guide the search for prospective sources, while the second purpose is to monitor the fractionation of a new plant, hopefully leading to the isolation of new anti-cancer drugs. The goal of the combined
efforts of fractionation and bioassay testing is to find compounds with high activity against cancer to test in more detail as potential clinically active drugs.

The bioassay testing system is divided into two types of testing methods. Probably the most reliable of these methods is the *in vivo* system because of its ability not only to test anti-cancer activity, but also to test toxicity. The evaluation of a potential drug by this system is accomplished by giving various dose levels to a group of mice into which have been implanted a specific tumor system and observing the lifetime of treated versus untreated mice. Currently the most common specific tumor systems are the P-388 (PS) or L-1210 leukemias. The evaluation is accomplished by expressing the lifetime of treated animals (T) and the lifetime of untreated animals (C) as a ratio \( \frac{T}{C} \times 100 \). When the "T/C" value is greater than 100 the potential drug is considered active, while when less than 100 the potential drug is considered toxic. For the potential drug considered active to be further investigated experimental experience of previous drugs tested at the biomedical laboratories have led to the National Cancer Institute to set a minimum "T/C" value of 125, with values greater than or equal to 150 considered good and prospective drugs normally having values in the 180-250 range.

This testing system has proved fairly reliable as a predictor of anti-cancer activity in man even though it has some disadvantages. It suffers the disadvantages of biological variation of individual animals, of requiring large amounts of the potential drug and of expense and slowness.
A second *in vivo* testing system, the Walker carcinosarcoma, was used in the early stage of testing the crude extracts, but was dropped by the National Cancer Institute for further use due to the better results of the PS testing system for evaluation of the potential drug.

The second testing method used is the *in vitro* cell culture system. This system involves the addition of increasing dose levels of a test sample to certain tumor systems growing in cell cultures until the growth is inhibited by fifty percent. The results of this system are expressed as \( \text{ED}_{50} \), in units of \( \mu \text{g/ml} \) relating to the dose level required for the inhibition of growth by fifty percent. The smaller the \( \text{ED}_{50} \) value, the more active the drug is considered as an inhibitor of cancer. Values equal to or less than 20 are arbitrarily considered positive indicators of cytotoxicity. The two cancer tumor cell systems are the P-388 leukemia which was also used in the *in vivo* test described earlier and tumor cells derived from Eagle's carcinoma of the nasopharynx which has been given the code letters "KB."21

The *in vitro* systems, although not as good predictors for clinical activity as the *in vivo* systems, have the advantages of requiring a smaller amount of potential drug and of being faster and more economical. The disadvantage of this system, that is readily apparent, is that in testing the cytotoxicity of a sample it does not test the toxicity of the sample towards normal cells. Because of the smaller amounts of sample required for testing, an *in vitro* system is used in the later fractionations for the isolation study where sample size is often limited. It is hoped that once an active drug is identified, it will also be active in the *in vivo* systems.
Previous Studies

*Ipomoea pes-caprae* has been the subject of two previous phytochemical investigations, by Cwalina and Jenkins\(^{22}\) and by Christensen and Reese.\(^{23}\)

Cwalina and Jenkins investigated the pharmacological value of various extractives administered to cats. They found no apparent action in their research study, but isolated the following from the extract: pentatriacontane, triacontane, a sterol \((C_{29}H_{49}OH)\), behenic acid, melissic acid, butyric acid and myristic acid. They also observed the presence of glycerides of saturated and unsaturated fatty acids, chlorophyll, inorganic salts, and a catechol tannin.

Christensen and Reese examined the antiseptic action of various leaf extractives. They found no noticeable pharmacological activity of the leaves or extracts, but isolated the following constituents: mucilage, a volatile oil, a complex resin, fat, a phytosterol, bitter substances, and a red coloring matter.

In a final study by Wasuwat\(^{24}\), it was found that a volatile fraction of *Ipomoea pes-caprae* was an antagonistic agent to histamine and jelly-fish poison, comparable to Benadryl (diphenhydramine-HCl) or Antistine (antazoline methane sulfonate).
RESULTS AND DISCUSSION

Extraction and Attempted Isolation of a Cytotoxic Component

The dried leaves, stems and roots of *Ipomoea pes-caprae* received were ground using a hammer mill into a fine powder. The extraction was then carried out using the following procedure.25 The ground plant was first extracted in a soxhlet extractor using hexanes for eight hours, and the hexane extract evaporated and stored. The ground plant was air dried and then extracted in the soxhlet extractor a second time using ethanol for eight hours. The plant was discarded after this step and the ethanol was concentrated in a rotary evaporator. The ethanol extract was initially separated into two portions at this point with the smaller one (Fraction A) dried for testing and the larger portion partitioned between chloroform and water. The water portion was discarded, since experience has indicated that this fraction contains few useful antineoplastic agents and the chloroform portion (designated fraction B) dried for chromatographic separations. These extraction procedures are summarized in Chart I.

The assay results from testing fractions A and B of the extraction are shown in Table I. Fractions A and B showed marginal activity only at high dose levels in the *in vivo* testing system using the P-388 tumor system, while the *in vitro* testing system showed only very slight cytotoxicity for both fractions. Although the ED$_{50}$ values of both fractions were larger than the value of 20 normally used to indicate cytotoxicity it was decided to continue purification of fraction B, the most cytotoxic fraction, in hopes that the activity might be concentrated.
Fractionation

Ground Plant (3.6 kg)

- Soxhlet, hexane, 8 hrs

  Plant

  - Air Dry
  - Soxhlet
  - EtOH 8 hrs

  - Plant discard
  - Ethanol evaporate

  Partition

  - H₂O/CHCl₃

  - H₂O discard
  - Chloroform evaporate

  Chloroform extract (50 g)

  Sample for assay

Chart I
Table I

Assay results on fractions from *Ipomoea pes-caprae*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>T/C (dose in mg/kg) P-388</th>
<th>ED$_{50}$ (µg/ml) P-388</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>118(400) 116(50) 124(25)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>B</td>
<td>130(200) 121(100) 118(50)</td>
<td>39</td>
</tr>
</tbody>
</table>
by further fractionation. To guide this fractionation the use of the
in vitro P-388 leukemia testing system was chosen because it was hoped
that the marginal in vivo P-388 activity observed would show up better
in the in vitro system.

The chloroform extract (Fraction B) was therefore subjected to
chromatography on a 6.5 cm silica gel open column using gradient elution
beginning with a chloroform/hexanes mixture, progressing to chloroform
and ending with a chloroform/methanol mixture as described in the
experimental section. Since the greatest amount of material was found
in three final fractions it was necessary to rechromatograph these
fractions. These fractions were combined and rechromatographed on
silica gel using an eluting solvent mixture of methylene chloride:
methanol: water to obtain further fractions. A summary of the two
column chromatographic fractionations can be found with the corresponding
bioassay test results in Chart II.

The fractions obtained by this procedure showed mild cytotoxicity
only in fraction 0. This fraction was accordingly subjected to
preparative thin layer chromatography with the solvent system methylene
chloride: methanol: water (91:8:1). Unfortunately none of the resulting
fractions showed as good cytotoxicity as the initial crude fraction 0.
The reason for this is not clear, but it is most likely due to either
biological variations in the testing method or sample decomposition.
In any event, since the cytotoxicity of fraction 0 was marginal, and
since the most active fraction from the preparative thin layer chroma-
tography was a small one, it was decided not to pursue the isolation of
the active component further at this time.
Chart II
Fraction B

Chromatography on silica gel gradient elution CHCl₃/hexane to CHCl₃/methanol

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
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<tbody>
<tr>
<td>m</td>
<td>1.5g</td>
<td>1.3g</td>
<td>0.7g</td>
<td>0.9g</td>
<td>1.4g</td>
<td>0.6g</td>
<td>1.5g</td>
<td>12.4g</td>
<td>13.1g</td>
<td>5.9g</td>
<td>1.4g</td>
</tr>
<tr>
<td>m</td>
<td>100</td>
<td>32</td>
<td>36</td>
<td>41</td>
<td>30</td>
<td>24</td>
<td>30</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
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Phthalate  β-sitosterol

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>O</th>
<th>P</th>
<th>Q</th>
<th>R</th>
<th>S</th>
<th>T</th>
<th>U</th>
<th>V</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>0.3g</td>
<td>1.9g</td>
<td>3.7g</td>
<td>2.5g</td>
<td>1.0g</td>
<td>0.5g</td>
<td>1.0g</td>
<td>0.9g</td>
<td>1.0g</td>
<td>1.1g</td>
</tr>
<tr>
<td>m</td>
<td>35</td>
<td>13</td>
<td>40</td>
<td>&gt;100</td>
<td>50</td>
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<td>&gt;100</td>
<td>&gt;100</td>
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PTLC

Fractions showed no activity

HPLC

Glycoside Z
0.02 g
>100
In view of the fact that the studies leading to the production of fraction O had produced a number of other partially purified fractions, it was decided to focus attention on the nature of the constituents of some of these fractions, in the expectation that interesting new compounds might be discovered. In part this expectation was realized and a new glycoside Z was isolated together with the common plant sterol, β-sitosterol, and a phthalate ester derived from the solvents used.

Isolation of Constituents

Isolation of Compound Y (β-sitosterol)

Fraction E from the column previously described yielded crystalline material directly in some collection tubes of the fraction collector, and the same material was shown by thin layer chromatography to be present also in fraction D. Crystallization of fraction D and recrystallization of fraction E were found to be best accomplished by using ether as the solvent. The crystalline material after appropriate recrystallization and combination for identification was designated compound Y.

Isolation of Glycoside Z

Fraction V from the second chromatographic separation previously described appeared by thin layer chromatography to consist of one major component. Attempts to crystallize the material failed to give any crystals and so preparative thin layer chromatography was used to purify the sample. This, however, did not help in the attempts at crystallization and so a final purification was made by high pressure liquid chromatography.
A number of solvent systems and column combinations was tried and it was found that the best separation was obtained with the system methanol: water, 85:15 on a Partisil ODS column. Under these conditions the partially purified fraction was shown to consist of one major component with several minor impurities. Preparative scale separation was achieved on a 1/2" x 50 cm Partisil 10 ODS column with the same solvent mixture. The major component was only freed from one particular impurity after four passes through the column, achieved by using the instrument in the recycle mode.26 (Figure 1). Under these conditions about 10 mg of crude sample could be purified per injection and a total of 50 mg was purified in all. This procedure yielded a compound designated Glycoside Z.

Isolation of Compound X (Bis-(2-ethylhexyl)Phthalate)

A fraction corresponding to fraction C from the first chromatographic separation previously described appeared to be largely homogeneous by thin layer chromatography. After precipitation of a waxy impurity with methanol, the filtrate was purified by preparative gas chromatography to yield as the major volatile component an oily material which was designated compound X.

Identification of Constituents

Beta-sitosterol26

Compound Y was a white crystalline material with a melting point range, 136-137°C. The ultraviolet absorption spectrum showed only end absorption indicating the absence of any chromophore in the molecule.
Identification of the compound as β-sitosterol was based on the properties discussed below.

**Carbon-Hydrogen Analysis**

The carbon-hydrogen analysis of compound Y gave the following observed values: C, 83.72, H, 12.01% as compared to the calculated values: C, 83.99, H, 12.15%. This information and the mass spectrum parent peak, m/e 414, were consistent with a C_{29}H_{50}O molecular formula.

**Optical Rotation**

The optical rotation of compound Y in chloroform gave an \([\alpha]_D\) value of \(-36^\circ\). The literature value for β-sitosterol is \(-37^\circ\)^{27}

**Infrared Spectrum**

The infrared spectrum of compound Y showed a broad hydroxyl band at 3410 cm\(^{-1}\). The other major features were alkane and alkene stretching and bending. The alkane C-H stretching was observed at 2965-2840 cm\(^{-1}\) and bending at 1385 cm\(^{-1}\). Unsaturation indicating the presence of a double bond was indicated by peaks at 3010-20, 1650 and 960 cm\(^{-1}\). With the exception of a small peak at 1735 cm\(^{-1}\) the infrared spectra of compound Y and authentic β-sitosterol are identical (Figures II).

**Proton Magnetic Resonance Spectrum**

The proton magnetic resonance spectrum of compound Y is shown in Figure III. In this spectrum can be seen the presence of a single proton resonance signal at δ5.4, a single proton with complex splitting at δ3.6 and an intricate aliphatic portion of the spectrum consisting of approximately fifty protons.
The single proton signal at 65.4 can be assigned to the proton at C-6 which would be split into a doublet of doublets by the adjacent methylene protons if it could be observed clearly. The complex splitting of the single proton signal at 63.6 is due to the splitting of the carbonyl proton by adjacent methylene protons. The aliphatic proton region can be partially elucidated by the identification of various methyl groups in beta-sitosterol.\(^{28}\) (1). The angular methyl groups, C-18 and C-19 were found at 1.0 and 0.67 ppm. The C-27 and C-26 isopropyl doublet was found at 0.88 and 0.77 ppm and approximately superimposed on the C-21 methyl group doublet. The C-29 methyl group protons appeared as an irregular triplet centered at 0.87 ppm.

**Mass Spectrum**

The mass spectrum of compound Y (Figure IV) showed a large parent molecule ion at \(m/e\) 414, which indicated a fairly stable structure. Two small peaks which occurred near the parent ion were due to the loss of a methyl group (\(m/e\) 399) and of a water molecule (\(m/e\) 396). There also appeared a small peak at \(m/e\) 381 (M-33) which resulted from either the initial loss of the methyl group followed by the loss of water or vice versa, or simultaneous loss of both groups.

The predominant feature of beta-sitosterol in this spectrum is the fragmentation series of a hydrocarbon steroid (\(m/e\) 55, 67, 81, 95 and 109). The other important features of this spectrum are the three common fragmentation patterns of beta-sitosterol (Chart III). Cleavage of ring B by a Retro-Diels-Alder process results in fragment b at \(m/e\) 273 composed of rings C and D and the side chain. The ring A portion
of the molecule yields ion \( a \) at \( m/e \) 138 by the same process and this ion can further decompose by loss of water to yield ion \( f \) at \( m/e \) 120. Rupture of ring C results in fragments \( c, d \) and \( g \) at \( m/e \) 251, 163 and 145 respectively of which fragment \( d \) is not observed probably due to decomposition in the ion source. The breaking of ring D and loss of the side chain leads to the formation of ion \( e \) at \( m/e \) 231 accompanied by the loss of water to give ion \( h \) at \( m/e \) 213. In the event of a double bond at \( C_5-C_6 \) in ring B, such is the case with \( \beta \)-sitosterol, fragmentation occurs which gives peaks at \( m/e \) 303 and \( m/e \) 111.\(^{29}\) Comparison with authentic \( \beta \)-sitosterol was carried out under identical conditions and the only variation was found to be the intensity of the fragment peaks.

Fraction Y was also compared to an authentic reference sample of \( \beta \)-sitosterol by thin layer chromatography. Both samples gave identical \( R_f \) values of 0.19 in a 1% methanol/chloroform solvent system.

**Glycoside Z**

Preliminary studies on the partially purified compound Z (fraction \( V' \)) were carried out by nuclear magnetic resonance and infrared spectroscopy. The proton magnetic resonance spectrum of fraction \( V' \) showed a distorted triplet at 60.9, which is presumably a methyl group at the end of a methylene chain and a strong signal at 61.2 together with other signals in the region 3.2-5.4 ppm. The infrared spectrum of fraction V showed a strong broad hydroxyl band at 3400 cm\(^{-1}\), C-H stretching at 2910 and 2840 cm\(^{-1}\) and a carbonyl band at 1740 cm\(^{-1}\). The rest of the spectrum gave very few clear adsorption peaks even in the "finger-print" region.
and of these, most were too broad to make any significant assignments. From this information plus consideration of compounds found in plants from the same family arose the possibility of the fraction being a glycolipid.\textsuperscript{30-34} The large peak at 61.2 could then be assigned to the methylene protons of a long chain carboxylic acid, while the peaks at 3.4-4.4 and 4.8-5.4 ppm could most naturally be assigned to the methine protons and hydroxyl protons respectively of a sugar moiety. The hydroxyl band in the infrared spectrum would, of course, be due to the hydroxyl groups of the sugar moiety and the carbonyl band due to the carboxyl group of the fatty acid.

In order to identify compounds of this nature it is necessary to cleave the two portions of the compound apart and carry out subsequent work on each portion separately. Two different procedures were used to achieve this step, both for their individual advantages and as checks on each other to be sure the reactions were responding as desired. The first procedure involved the use of HCl-MeOH to effect the cleavage, which yields the methyl ester derived from the carboxylic acid moiety. The second procedure involved the use of trifluoroacetic acid to hydrolyse the sample; this is a milder reaction used especially for identification of the sugar moiety, but is also good for giving the free fatty acid.

The hydrolysis reaction using 50 mg of fraction V was evaluated by thin layer chromatography with a CH\textsubscript{2}Cl\textsubscript{2}/MeOH/H\textsubscript{2}O (90:9:1) solvent system. Detection by the sulfuric spray reagent followed by heating showed two new spots not present in the unreacted sample. Some of the hydrolysed sample was then methylated using diazomethane. Thin layer chromatography showed the disappearance of the hydrolysis products and
the gas chromatograph using an SE-30 column programmed for 75°-250°C at 8°C/min. showed two major peaks in a 1:2 ratio. These results were slightly questionable since this hydrolysis was carried out on a crude sample, fraction V, which presumably contained the glycosides of two different fatty acids. Using fairly specific spray reagents, bromo-cresol purple sodium salt and silver nitrate-ammonium hydroxide-sodium methoxide the presence of a fatty acid and a sugar in the hydrolysate was indicated.35 These results support the initial assumption that the fraction is a glycolipid. Additional evidence in support of this fact was found by testing for the sugar. The sample prior to hydrolysis gave a negative test on treatment with Benedict's solution, but after hydrolysis gave positive results both for the Benedict's and Molisch test.36 Since the Benedict's test works only for reducing sugars the only way to prevent a reducing sugar from giving a positive Benedict's reaction would be through the union to some other compound via a glycosidic linkage. Finally, a test of unhydrolysed compound by thin layer chromatography followed by Bromocresol purple-sodium salt spray reagent indicated the compound contained a free carboxyl group.

The evidence to this point, obtained largely on the partially purified material, fractions V and V', indicates that the material consists of a long-chain fatty acid bonded through a glycosidic linkage to a sugar moiety. Since the carboxyl group of the acid is free (IR and TLC spray reagent evidence) it follows that it must bear at least one hydroxyl group through which linkage to the sugar can be made. The problem of the structure elucidation of Glycoside Z, thus, resolves itself into the
problems of the elucidation of the structures of the acid and sugar portions and then the determination of the nature of the linkage between them.

Analysis of the Fatty Acid

Methanolysis of fraction V (HCl/MeOH) yielded a crude product which after appropriate purification was shown by gas chromatographic analysis using an SE-30 column programmed for 150-230°C at 2°C/minute to consist of one major volatile component. The purified compound Z was also converted to its methyl ester by hydrolysis (TFA) followed by methylation and the same major volatile material was produced by this procedure. All subsequent work was, thus, carried out on the major fraction obtained from the partially purified material, fraction V', designated ester AA.

The retention time of ester AA under the standard conditions noted above was intermediate between those of methyl myristate and methyl stearate, indicating a chain length of 15 or 16 carbons. A sample of ester AA was obtained in pure form by preparative gas chromatography and its spectra determined and analysed as discussed below.

Infrared Spectrum

The infrared spectrum of the ester AA shows three strong peaks at 2920, 2840 and 1725 cm\(^{-1}\) and some medium strength peaks at 3340, 1430, 1405, 1255, 1195, 1170 and 810 cm\(^{-1}\). The peaks at 2920, 2840 and 1430 cm\(^{-1}\) can be assigned to alkyl stretching and bending and the 1725 cm\(^{-1}\) peak which appears to have a shoulder can be attributed to the methyl ester functional group. The peak at 3340 cm\(^{-1}\) can be due to the
hydroxyl group which though at a little lower wavelength can be explained by intra and inter-hydrogen bonding.

**Proton Magnetic Resonance Spectrum**

The proton magnetic resonance spectrum of the methoxy fatty acid methyl ester, ester AA, is shown in Figure V. In this spectrum one notices a three proton triplet signal centered at 60.9, a large signal with a few shoulders on it at 61.3, a two proton triplet at 62.3 and a superimposed singlet over a multiplet at 63.6. The triplet signal centered at 60.9 is the result of a methyl group signal split by the adjacent methylene protons and the large signal at 61.3 is due to the methylene protons occurring at essentially the same chemical shift. The two proton triplet signal at 62.3 occurs due to the splitting of the methylene protons adjacent to the ester by its neighboring methylene group into a triplet. The superimposed signals at 63.6 are those of the methoxy protons giving a 3 proton singlet and the carbinol proton split by adjoining methylene groups into what would be a quintet. Signals just below 1.3 ppm are assigned to methylene protons adjacent to the carbinol carbon.

**Mass Spectrum**

The mass spectrum of the fatty acid methyl ester (Figure VI) did not show a very strong parent molecular ion peak as would be expected for a hydroxy-fatty acid methyl ester. The loss of water and water with either a methoxy group or methanol at m/e 268, 237 and 236 respectively suggested that the parent molecular ion peak would be at m/e 286 and a small peak is detectable at this position. The predominant fragmentation
pattern is the cleavage on both sides of the hydroxyl group to yield ions at \( m/e \) 101 and 215. The ion at \( m/e \) 215 undergoes further fragmentation by loss of methanol to yield an abundant ion at \( m/e \) 183 and this fragmentation is supported by the observation of a metastable peak at \( m/e \) 156 (calculated for 215 \( \rightarrow \) 183 is 155.8). The ion at \( m/e \) 215 also undergoes loss of CHO to yield an ion at \( m/e \) 186. McLafferty rearrangement of the initial ester yields the expected peak at \( m/e \) 74. These fragmentations are summarized in Chart IV and in conjunction with the nuclear magnetic resonance results provide conclusive proof that the structure of ester AA is that of methyl \( \eta_{11} \)-hydroxyhexadecanoate. \( \eta_{11} \)-hydroxyhexadecanoic acid has been isolated previously from plant sources and is known as jalapinolic acid.

Upon a literature search this acid was found to have such a small optical activity as to have been reported at one time optically inactive. This precluded any attempt with the limited quantity available of obtaining the optical rotation of the ester which was also very small, but it may reasonably be assumed that the isolated material has the same chirality as samples isolated from other plant sources (+).

Identification of the Carbohydrate Moiety

The first procedure used for the identification of the carbohydrate portion of the glycoside was the formation and gas chromatography-mass spectrometric analysis of the alditol acetate derivative. The alditol acetate derivative was prepared by using the aqueous portion of the chloroform/water partitioning of the trifluoroacetic acid hydrolysis and taking it through the series of reactions outlined below (Chart V).
Alditol Derivatization

\[
\text{CH}_3(\text{CH}_2)_4\text{CH}(\text{CH}_2)_9\text{COOH} \xrightarrow{2\text{N CF}_2\text{COOH} \sim 76^\circ\text{C}} \text{CH}_3(\text{CH}_2)_4\text{CH}(\text{CH}_2)_9\text{COOH} + \]

\[
\text{CH}_3\text{CH}_2\text{OH} \xrightarrow{0.8\text{M NaBH}_4} \text{CH}_3\text{CH}_2\text{OAc}
\]

\[
\text{H-C-0H} \xrightarrow{\text{Ac}_2\text{O, NaOAc}} \text{H-C-0Ac}
\]

Chart V
The sugar was reduced with sodium borohydride from the aldehyde form to the alcohol form and then acetylated using acetic anhydride and sodium acetate. The resulting alditol acetate derivative was analyzed by gas chromatography on an OV-225 column. The retention time from the gas chromatograph indicated the possibility of the sugar being either fucose or rhamnose. The mass spectrum shown in figure VII showed the highest mass peak at \( m/e \) 361 with major fragment peaks corresponding to the repetitive loss of 72 mass units occurring at \( m/e \) 289, 217, 145 and 73. This fragmentation pattern, plus the absence of peaks at \( m/e \) 303, 231, 159 and 87 resulting from a terminal methyl group, suggested that the parent molecular ion peak would be at \( m/e \) 434, which was not observed (Chart VI). This information was contradictory to the gas chromatographic retention times which suggested the sugar moiety was fucose or rhamnose (\( m/e \) 376) that have terminal methyl groups. Because of the apparent contradiction, it was decided to make the aldononitrile acetate derivative. The aldononitrile acetate derivative gives single, fairly well separated gas chromatographic peaks and under proper mass spectrometric conditions the parent molecular ion peak is observed which would resolve the conflicting results.\(^{41-42}\)

The aldononitrile acetate derivative was formed by treating the same dried aqueous portion (TFA hydrolysis) as used for preparing the alditol acetate derivative with hydroxylamine hydrochloride in pyridine followed by acetylation using acetic anhydride.\(^{43-44}\) (Chart VII) Comparison of gas chromatographic retention times of the unknown aldononitrile acetate derivative with seven known aldononitrile acetate derivative standards showed a major sugar component corresponding to
Chart VI
Aldononitrile Derivatization

\[
\text{CH}_3(\text{CH}_2)_4\text{CH}(\text{CH}_2)_9\text{COOH} \rightarrow \text{CH}_3(\text{CH}_2)_4\text{CH}(\text{CH}_2)_9\text{COOH}
\]

Chart VII
rhamnose and a minor sugar component corresponding to fucose. The chemical ionization mass spectra of the two sugar moieties showed (M+ +1) parent molecular ion peaks at m/e 330, which is in agreement with the assignments of rhamnose and fucose. (Figures VIII & IX). These chemical ionization spectra showed a prominent ion at m/e 270 corresponding to the loss of CH$_3$COOH and a smaller ion at m/e 300 corresponding to the loss of HCN. The electron impact spectra showed the loss of CH$_2$OAc and CH(OAc)$_n$CN fragments by $\alpha$-cleavage of the alditol chain. (Figures X & XI).

Quantitative evaluation of the number of sugars attached to the fatty acid was not possible due to dilution of bleeding the reverse phase column. Thus, the evaluation was made by comparison of the integration from the proton magnetic resonance spectrum of the glycoside with the integration expected for a mono- and disaccharidic glycoside, Table II, which shows the observed integration to be more consistent with a monosaccharide.

Since the presence of both rhamnose and fucose were both firmly established and the glycoside is monosaccharidic, then "Glycoside Z" must consist of a mixture of two glycosides whose sugar moieties differ. These glycosides were not separated, presumably because the sugar moieties are closely related to each other and since a small difference

<table>
<thead>
<tr>
<th>315</th>
<th>242</th>
<th>171</th>
<th>99</th>
<th>28</th>
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<td>H</td>
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<td>H</td>
<td>H</td>
<td></td>
</tr>
<tr>
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<td>G</td>
<td>G</td>
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</tr>
<tr>
<td>OAc</td>
<td>OAc</td>
<td>OAc</td>
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<tr>
<td>14</td>
<td>87</td>
<td>159</td>
<td>231</td>
<td>303</td>
</tr>
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</table>
Figure IX.
(Fucose CI)
Table II

Glycosidic Proton Magnetic Resonance Integration Comparison

# protons

<table>
<thead>
<tr>
<th>Structure</th>
<th>Obs.</th>
<th>Mono-</th>
<th>Di-</th>
</tr>
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<td>3</td>
</tr>
<tr>
<td>CH₂CH-0</td>
<td></td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>-(CH₂)n-</td>
<td></td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>CH₂COOH</td>
<td>3.9</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>-CH-O-</td>
<td></td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>-CH-OH</td>
<td></td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Total Obs. = 25.8


in the sugar portion would make only a minor difference in the physical and chemical properties of the overall molecule.

Because of the limited quantity of "Glycoside Z," a determination of the anomeric configuration of the linkage between the fatty acid and sugar portions was not attempted, but previous studies of glycosides have indicated that the normal attachment has been the $\beta$-configuration of the sugar-fatty acid linkage.

The two glycolipids, previously described as Glycoside Z, are thus identified as $\beta$-rhamnopyranosyl-11-hydroxyhexadecanoic acid and $\beta$-fucopyranosyl-11-hydroxyhexadecanoic acid whose structures are shown below.

\[
\text{HO CH O H} \quad \text{CH}_3 \\
\text{CH}_2 \quad \text{CH} \\
\text{OH OH OH} \\
\text{OH OH OH}
\]

\[
\text{CH}_3 \\
\text{CH}_2 \quad \text{CH} \\
\text{OH OH OH} \\
\text{OH OH OH}
\]

In spite of the fact that resin glycosides have been known and studied for a long time, few detailed structural investigations of resin glycosides of plant origin have appeared. Of these the methyl esters of $\beta$-rhamnopyranosyl-11-hydroxyhexadecanoic acid and $\beta$-fucopyranosyl-11-hydroxyhexadecanoic acid have previously been found in Ipomoea fistulosa. As of present, however, $\beta$-rhamnopyranosyl-11-hydroxyhexadecanoic acid and $\beta$-fucopyranosyl-11-hydroxyhexadecanoic acid have not been reported and appear to be new compounds.
Bis (2-ethylhexyl) Phthalate

Compound X, a liquid material purified by gas chromatography, was identified by the spectroscopic methods discussed below.

Infrared Spectrum

The infrared spectrum of compound X showed a very strong carbonyl band at 1745 cm\(^{-1}\). The other strong bands were observed at 2980, 2940, 2880, 1300, 1140 and 1090 cm\(^{-1}\) with two medium intensity bands at 1480 and 755 cm\(^{-1}\). The strong carbonyl band could be assigned to ester stretching with peaks at 1300 and 1140 cm\(^{-1}\) suggesting that the ester is adjacent to a double bond or aromatic ring. The bands at 2980, 2940 and 2880 cm\(^{-1}\) could be assigned to alkane stretching and the peaks at 1480 and 755 cm\(^{-1}\) corresponded to a substituted aromatic ring structure.

Ultraviolet Spectrum

The ultraviolet spectrum showed two absorption peaks at 226 and 276 nm with absorption values of 6.9 \(\times\) 10\(^3\) (log \(\varepsilon\) 3.84) and 990 (log \(\varepsilon\) 2.99). These values confirm the belief that compound X was a substituted aromatic ester.

Mass Spectrum

The mass spectrum of compound X (Figure XII) showed a parent molecular ion peak at \(m/z\) 390 with the loss of M-29 and M-57 showing small ion peaks. The major ion peak at \(m/z\) 149 indicated the compound was a phthalate.
Nuclear Magnetic Resonance

The proton magnetic resonance spectrum of compound X is shown in Figure XIII. The spectrum shows the presence of four aromatic protons at 64.08, an eighteen proton multiplet at 61.3 and a twelve proton pair of triplets at 60.92. From this information plus previous spectroscopic data it may be deduced that compound X is a symmetrically disubstituted benzene ring having two ester groups with the structure \(-\text{CH}_2\text{CH}_2-\) adjacent to them and four groups with the structure \(\text{CH}_3\text{CH}_2-\).

Upon inspection of the proton decoupled cmr spectrum (Figure XIV) several features of the structure were immediately clarified. The carbonyl group was apparent from the signal at 167.4 ppm and the three signals about 130 ppm indicated compound X was an ortho-disubstituted benzene ring in contrast to meta and para substituted benzenes which would give four and two signals respectively. The methylene group adjacent to the ester was also evident at 67.8 ppm. The seven other carbons in the range 39-10 ppm could only be further distinguished by using coherent off-resonance proton decoupling (Figure XV).

Coherent off-resonance proton decoupling is a technique which is accomplished by off-setting the proton irradiation frequency from exact resonance and reducing the irradiation power, whereby residual coupling for carbons by directly attached protons is observed. This enables the assignment to be made of the signals at 10.9 and 13.9 ppm which appear as quartets to methyl carbons. The signals at 22.9-30.3 ppm though a bit ambiguous appear like two sets of two overlapping triplets. The signals at 38.7 ppm was split into a doublet resulting from a methine carbon and the other previously assigned resonances were additionally
confirmed by their splitting. The methylene group adjacent to the ester split into a just detectable triplet, the aromatic carbons split into two doublets and a singlet and the carbonyl carbon remained an unperturbed singlet.

By narrow band irradiation of specific proton type resonances, coherent proton decoupling could be obtained of the corresponding carbons. This technique showed the signals at 10.9 and 13.9 ppm in the cmr spectrum appeared as singlets while other aliphatic carbons appeared as multiplets when the methyl protons were irradiated.

With the evidence obtained by nuclear magnetic resonance and the molecular weight alone compound X was shown unambiguously to contain the following structural elements, where X indicates an electronegative group.

\[
\begin{align*}
\text{H} & \quad -\text{CH}_2- \quad -\text{CH}_2\text{CH}_3 \\
\text{C} & \quad \text{X-CH}_2\text{CH-} \quad -\text{CH}_2- \quad -\text{CH}_2\text{CH}_3 \\
\text{H} & \quad -\text{CH}_2 \quad -\text{CH}_2\text{CH}_3 \\
\text{C} & \quad \text{X-CH}_2\text{CH-} \\
\text{O} & \quad -\text{CH}_2- \quad -\text{CH}_2\text{CH}_3
\end{align*}
\]

It was possible to distinguish the two possible structural formulas (I and II) on the basis that structure II would show only nine different carbons whereas structure I would show the observed twelve different carbon resonances. Thus, the structure of compound X is that of structure I, which is known as bis(2-ethylhexyl)phthalate.
Since bis(2-ethylhexyl)phthalate is a well known plasticizer another extraction was carried out avoiding all contact with any plastics. This compound was found to be still present after extraction and further separation. Examination of the reagent grade solvents used gave residues upon distillation and of these the phthalate was found to be present in chloroform. This has led to the distillation of all solvents excluding ether prior to using them for this type of research.
As stated at the beginning of this thesis, the primary goal of the research was to isolate and characterize an active anti-cancer compound from *Ipomoea pes-caprae* and the secondary goal was to isolate and characterize other constituents of the plant. Although the primary goal could not be reached, for reasons which have been described and which relate primarily to the low activity of the initial plant sample, it was possible to reach the secondary goal. The isolation of the known plant steroid, β-sitosterol and the isolation and characterization of two unusual glycolipids, β-rhamnopyranosyl-11-hydroxyhexadecanoic acid and β-fucopyranosyl-11-hydroxyhexadecanoic acid is described. In addition, the observation was made that phthlate plasticizer can contaminate solvents even before they reach the laboratory and an analysis of the $^{13}$C magnetic resonance spectrum of bis-(2-ethylhexyl) phthalate was carried out.

In conclusion, the lack of significant anti-cancer activity in *Ipomoea pes-caprae*, suggests that further investigation for anti-cancer compounds is not justified, and it is unlikely that *Ipomoea pes-caprae* contains any anti-cancer compounds of potential clinical use.
EXPERIMENTAL

General Information

Solvents used throughout this research were reagent grade. Except where otherwise stated, all solvents were distilled before use.

Analytical and preparative thin layer chromatography plates were made in the laboratory by spreading an aqueous slurry of silica gel with a Shandon UNOPLAN mechanical spreader. The analytical plates, usually 5 x 20 cm, were made of E. Merck silica gel GF-254 with a 0.30 mm layer thickness setting. The preparative plates, 20 x 20 cm, were made from E. Merck silica gel 60 PF-254 spread to a thickness of 1.0-1.5 mm. Visualization of thin layer chromatography plates was accomplished by using a Chromato-Vue CC-20 long and short wavelength viewing apparatus manufactured by Ultra-violet Products, Inc. Further visualization was performed by spraying with anisaldehyde-sulfuric acid solution (1 ml sulfuric acid, 0.5 ml anisaldehyde, 50 ml acetic acid) or treatment with iodine vapor.

Gas chromatographic separation and analyses were performed with a Varian Aerograph Series 2740 Moduline instrument with a flame ionization detector. Columns used were an analytical SE—30 100/200 Varaport 1/8" x 5', a preparative 10% OV-1 60/80 Chromosorb W 1/4" x 5' and an analytical 3% OV-225 1/8 x 5' glass column.

High pressure liquid chromatographic separation and analysis were performed using a Waters M-6000A high pressure pump and a Waters Associates refractive index detector. Columns used were prepacked normal phase and ODS columns from Reeve Angel. Other specifications for the gas chromatography and high pressure liquid chromatography are mentioned in specific applications.
Infrared spectra were either recorded in KBr or CHCl₃ on a Beckman IR-20AX infrared spectrometer.

Ultraviolet spectra were run in 95% ethanol solution on a Cary-14 ultraviolet-visible spectrometer.

Nuclear Magnetic Resonance spectra were obtained on a JEOLCO JNM-PS-100 instrument using deuteriochloroform as the solvent unless otherwise stated. Tetramethylsilane (TMS) was used as the internal standard.

The mass spectra were obtained on a Varian MAT-112 or a Hitachi Perkin-Elmer RMU-7 double focussing spectrometer. Perfluorokerosene was used as the reference for counting the spectra.

The carbon-hydrogen analysis were obtained on a Perkin-Elmer 240 Elemental Analyzer.

The optical rotation data were obtained using an O.C. Rudolph & Sons Inc. Model 70 polarimeter.

Extraction

The stems, roots and leaves of *Ipomoea pes-caprae* (PR 41615, NSC B636427) received from Panama through the U. S. Department of Agriculture were ground using a 1/4" mesh in a hammer mill to give totally 11.5 kilograms of which 300.0 grams was extracted immediately for bioassay testing and the remaining amount extracted in 4 portions. The extraction was carried out using a soxhlet extractor by putting approximately 1 kilogram of ground plant wrapped in cheese cloth into the extractor, filling the round bottom flask two-thirds full of the solvent and turning on the heating mantle and water to the condenser.
The ground plant was first extracted with hexanes for eight hours after which the hexane extract was concentrated and stored. The plant material was air dried overnight followed by soxhlet extraction using 95% ethanol for eight hours. The marc was discarded and the extract was concentrated in vacuo. The extract was next partitioned between chloroform and water (1:1) by adding small amounts of chloroform to dissolve the paste and then using water. In the cases when an emulsion formed between the two solvents, sodium chloride was added to salt out the chloroform in the aqueous layer. The chloroform layer, fraction B, was concentrated in vacuo for further separatory work. The water layer was discarded and some insoluble material which was between the two layers was stored. The Mayer's test\textsuperscript{47} for alkaloids was tried and gave a negative response indicating no alkaloids were present in the plant at the level at which this test is sensitive (approximately 0.01%).

Column Chromatography of Fraction B

The column packing procedure was as follows: 1,700 g of silica gel-60 (70-230 mesh) was dried in a 4 liter beaker in an oven at approximately 100°C for nine hours. It was deactivated by vigorously shaking with 10% distilled water (v/w) in 2 separate 4 liter flasks. This was done to prevent irreversible adsorption resulting from absence of water and poor separation from inadequate adsorption due to too much water.\textsuperscript{48} The slurry for packing the column was made by exponentially diluting the silica gel with 5 liters of 10% chloroform/hexanes with vigorous shaking, following by equilibration for 12 hours.
The column used for this chromatogram was 6.5 x 140 cm with glass wool in the bottom covered by sand. To prevent air bubbles from forming in the glass wool approximately 100 ml of the 10% chloroform/hexanes mixture was added prior to pouring in the slurry. Sand was added to prevent the glass wool from floating as it does in this solvent system. To insure both of these precautions were effective the glass wool and sand were pressed with a flex-a-fram rod with a supporting foot attached to it. After shaking the slurry to suspend all the silica gel into solution all the slurry was added in one continuous pouring which filled the column to capacity. The stopcock was opened to give an initial flow rate of 1 drop every 3-4 seconds. After letting the silica gel settle and pack the solvent was run through the column for about 12 hours.

The sample charge was prepared by adsorbing 45 grams of Fraction B on 140 grams of silica gel by dissolving the sample in chloroform, adding silica gel and drying in vacuo on a rotary evaporator. At this point the solvent head on the column was removed by pipetting until 1-2 cm of solvent remained. Filter paper cut to fit the inside diameter was placed on top of the column and then the sample charge was added followed by enough solvent to wet it thoroughly. Filter paper and sand were then added to the top of the column to prevent disturbing the sample when adding solvent.

Elution was carried out by starting with 8 liters of 10% chloroform/hexanes followed by 23 liters of chloroform, 4 liters of 5% methanol/chloroform, 6 liters of 10% methanol/chloroform and 10 liters of 30% methanol/chloroform.
Samples were collected and monitored by thin layer chromatography and the weight of fractions and combinations made to give the fractions found in Table III.

Column Chromatography of Combined Fractions J, K & L

For the column chromatography of combined fractions J, K & L several solvent systems were tested by thin layer chromatography in order to find the system of choice. Such solvent systems included mixtures of butanone with benzene or cyclohexane and mixtures of methanol-water with methylene chloride or ethyl acetate. The methylene chloride:methanol:water system appeared to give the best separation so the composition of the mixture was adjusted until the best ratio of methylene chloride:methanol:water was found to be 91:8.8:0.2.

The column used for this separation was 6.5 x 140 cm and filled with a slurry of 1 kilogram of Silica gel-60 (70-230 mesh) in 2 liters of the solvent. With the exceptions noted below the column chromatographic preparation was carried out the same as for fraction B previously mentioned. Cotton was used to form a small plug above the stopcock and a matt of cotton was put at the bottom of the column instead of glass wool. The flex-a-frame red and foot was used again to hold down the cotton in this case. The slurry was poured and after settling the sample charge was applied, using a pipette to add 31 grams of sample dissolved in approximately 150 ml of solvent. After the sample was adsorbed onto the silica gel more silica gel was added to the top of the column and solvent slowly added. As with the previous column a fraction collector was placed under the column and collection begun when the
<table>
<thead>
<tr>
<th>Fraction</th>
<th>Fraction No.</th>
<th>Fraction Wts.</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>G (F044)</td>
<td>1-40-1</td>
<td>0.398</td>
<td>Chloroform</td>
</tr>
<tr>
<td></td>
<td>1-40-2</td>
<td>0.451</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-3</td>
<td>0.533</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-4</td>
<td>0.084</td>
<td></td>
</tr>
<tr>
<td>D (F045)</td>
<td>1-40-5</td>
<td>0.503</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-6</td>
<td>0.756</td>
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</tr>
<tr>
<td>E (F046)</td>
<td>1-40-7</td>
<td>0.720</td>
<td></td>
</tr>
<tr>
<td>F (F047)</td>
<td>1-40-8</td>
<td>0.057</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-9</td>
<td>0.250</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-10</td>
<td>0.580</td>
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</tr>
<tr>
<td>G (F048)</td>
<td>1-40-11</td>
<td>1.098</td>
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<tr>
<td></td>
<td>1-40-12</td>
<td>0.302</td>
<td></td>
</tr>
<tr>
<td>H (F049)</td>
<td>1-40-13</td>
<td>0.331</td>
<td>5% MeOH/CHCl₃</td>
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<tr>
<td></td>
<td>1-40-14</td>
<td>0.256</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-15</td>
<td>0.061</td>
<td></td>
</tr>
<tr>
<td>I (F050)</td>
<td>1-40-16</td>
<td>0.204</td>
<td>10% MeOH/CHCl₃</td>
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<tr>
<td></td>
<td>1-40-17</td>
<td>0.255</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-40-18</td>
<td>1.080</td>
<td></td>
</tr>
<tr>
<td>J (F051)</td>
<td>1-40-19</td>
<td>12.43</td>
<td></td>
</tr>
<tr>
<td>K (F052)</td>
<td>1-40-20</td>
<td>13.12</td>
<td>30% MeOH/CHCl₃</td>
</tr>
<tr>
<td>L (F053)</td>
<td>1-40-21</td>
<td>5.962</td>
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</tr>
<tr>
<td>M (F054)</td>
<td>1-40-22</td>
<td>0.418</td>
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</tr>
<tr>
<td></td>
<td>1-40-23</td>
<td>1.016</td>
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</table>
first colored band was about two-thirds down the column. Fractions were combined on the basis of their thin layer chromatographic characteristics as shown in Table IV.

Isolation of Glycoside Z

Purification of Glycoside Z was first attempted using preparative thin layer chromatography using approximately 75 mg of fraction V with a (CH₂Cl₂/MeOH/H₂O 80:19:1) solvent system which gave one major and one minor band.

Further purification of Glycoside Z was accomplished by using reverse phase High Pressure Liquid Chromatography. Analytical separations showed the best system to be a methanol:water mixture, 85:15, on a 1/4" x 25 cm. Partisil ODS column with a flow rate of 2 ml/min, an attenuation setting of 16 and the detector set for negative polarity. Preparative scale separation was attained on a 1/2" x 50 cm. Partisil 10 ODS column using the same solvent mixture with increased flow rate of 4 ml/min, at an initial attenuation 32 with a sample load of 50 mg per injection. Purified sample was achieved by recycling the sample through the column three times before collecting. Approximately 5 mg of homogeneous material was obtained per injection.

Acid Cleavage of Glycoside Z

The cleavage of Glycoside Z was achieved by the use of two different hydrolysis procedures.

A. The partially purified sample, fraction V', (100 mg) was refluxed for 3 hours with 10% methanolic hydrogen chloride (5 ml). The hydrolysate was diluted with water (10 ml) and extracted (20 ml per
Table IV

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Fraction Nos.</th>
<th>Fraction Weights</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (F055)</td>
<td>1-52-(1-7)</td>
<td>0.321</td>
<td>875 ml.</td>
</tr>
<tr>
<td>O (F056)</td>
<td>1-52-(8-14)</td>
<td>1.969</td>
<td>750</td>
</tr>
<tr>
<td>P (F057)</td>
<td>1-52-(15-21)</td>
<td>3.75</td>
<td>750</td>
</tr>
<tr>
<td>Q (F058)</td>
<td>1-52-(22-28)</td>
<td>2.84</td>
<td>750</td>
</tr>
<tr>
<td>R (F059)</td>
<td>1-52-(29-32)</td>
<td>0.960</td>
<td>375</td>
</tr>
<tr>
<td>S (F060)</td>
<td>1-52-(33-37)</td>
<td>0.493</td>
<td>500</td>
</tr>
<tr>
<td>T (F061)</td>
<td>1-52-(38-45)</td>
<td>1.05</td>
<td>875</td>
</tr>
<tr>
<td>U (F062)</td>
<td>1-52-(46-53)</td>
<td>0.920</td>
<td>875</td>
</tr>
<tr>
<td>V (F063)</td>
<td>1-52-(54-62)</td>
<td>1.01</td>
<td>1000</td>
</tr>
<tr>
<td>W (F064)</td>
<td>1-52-(63-67)</td>
<td>1.15</td>
<td>500</td>
</tr>
</tbody>
</table>
The ether extracts, containing the methyl ester of the acid portion of the glycoside, were combined, washed, dried and evaporated to yield crude methyl ester (15 mg). The ester was purified by preparative gas-liquid chromatography (using a linear temperature program of 175-230°C at a rate of 2°C/min. with a carrier gas flow rate of 45 ml/min.) to yield chromatographically homogeneous material. This procedure was done twice to obtain 3 mg of the chromatographically homogeneous material.

B. The sample, Glycoside Z, (5 mg) was heated at 90° for 6 hours in a Microflex reaction vial with 2N trifluoroacetic acid (1 ml). The hydrolysate was dried over KOH in a vacuum desiccator, and then partitioned between water and chloroform. The aqueous extract containing the carbohydrate portion was treated as described below. The chloroform soluble portion was methylated with diazomethane to yield an ester identical to that obtained from the large scale hydrolysis A.

Preparation of Carbohydrate Derivatives

A. Alditol Acetate

The dried aqueous fraction from the trifluoroacetic acid hydrolysis described (approximately 2 mg) was treated with 0.4 ml of 0.8 M NaBH₄ solution at room temperature for 3 hours. The sodium borohydride was neutralized after the reaction with glacial acetic acid, and the acidified solution extracted with chloroform (1 ml) three times to remove any remaining lipid material and then blown dry using nitrogen. Methanol (1 ml) was added to the sample and blown dry using nitrogen four times. The sample was next reacted with 0.3 ml acetic anhydride
and 1 mg sodium acetate in a microflex reaction vial at 100°C for 4 hours. After letting the sample cool to room temperature, 1 ml of toluene was added and blown dry, three times. Finally the sample was partitioned between 1 ml chloroform and 1 ml of ion-free water and the chloroform portion dried and stored in a reaction vial at 0°C until injected into the gas chromatograph-mass spectrometer. The gas chromatograph was set up with an OV-255, 3% on 80-100 mesh column with a temperature program of 170°C-235°C at a rate of 2°C/min. and carrier gas flow rate of 45 ml/min.

B. Aldononitrile Acetate

The dried sugar sample (2-3 mg) was reacted with 5 mg of dried hydroxylamine hydrochloride in 5-6 drops of pyridine in a reaction vial at 90°C for 45 minutes. After cooling to room temperature, 15 drops of acetic anhydride were added, the vial resealed and heated at 90°C for another 45 min. period. The sample was next dried and partitioned between 1 ml nanograde chloroform and 1 ml of 3N HCl. The chloroform portion was washed with distilled water twice and once with 0.5 N NaHCO₃ followed by water again. The chloroform solution was finally dried using Na₂SO₄, filtered and stored at 0°C until injection in the gas chromatograph-mass spectrometer. The sample was injected into a gas chromatograph with an OV-225/2.50% glass column which was run isothermally at 215°C with a carrier gas flow rate of approximately 45 ml/min.
BIBLIOGRAPHY


9. Ibid., p. 143.


47. Suppliers No. and Acquisition No. for Ipomoea pes-caprae.


The vita has been removed from the scanned document
A study of the constituents of *Ipomoea pes-caprae* L.R. Br. was made as a part of a systematic attempt to isolate anti-cancer compounds of natural origin. Initial extraction and fractionation of the plant material failed to yield any fractions with significant *in vivo* activity in the P-388 tumor system, and it was concluded that the plant is not a good source of active anti-cancer compounds.

A study of the secondary metabolites of the plant revealed the presence of β-sitosterol and a complex mixture of glycolipids. Two components of the glycolipid mixture were characterized as β-rhamnopyranosyl-11-hydroxyhexadecanoic acid and β-fucopyranosyl-11-hydroxyhexadecanoic acid. In addition, the plasticizer bis-(2-ethylhexyl)phthalate was identified as a contaminant of certain batches of chloroform used in this work.