Analysis of Extractables from One Euphorbia

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ABSTRACT

Chemical analyses have been made of the heptane extractable material of *Euphorbia lathyris*, a plant which has been proposed as an "energy farm" candidate. The heptane extract is 4-5% of the dry plant weight and has a heat value of ~ 18 x 10^3 BTU/lb. This reduced photosynthetic material consists almost entirely of polycyclic triterpenoids.

INTRODUCTION

The growing of green plants for energy and chemicals has received much discussion, particularly the use of biomass as a renewable resource for fuel or for chemical feedstocks (1). More recently Buchanan and coworkers evaluated several hundred plant species as "energy farm" candidates (2,3). *Euphorbia lathyris* was identified as one of the few potential hydrocarbon-producing crops (4), and it has been successfully cultivated in California (Sachs, R., Department of Environmental Horticulture, University of California, Davis, private communication).

Reduced photosynthetic material can be obtained from the dried plant material by simple solvent extraction techniques. Various solvents can be used to extract different plant constituents; two schemes are shown in Figure 1. Direct heptane extraction yields a hydrocarbon like fraction which is 4-5% of the dry weight of the plant. This fraction has a heat value of ~ 18 x 10^3 BTU/lb (5) and is equivalent to the heptane soluble portion of the acetone extract. The high heat value and low oxygen content of this extract warranted an effort to investigate its chemical composition in detail.

In this paper we report on the identification of some major components of this plant extract. The nature of the methanol extractables and the more polar part of the acetone extract will be discussed separately.

METHODS

Extractions of Dried Plants

The reduced organic material is not uniformly distributed throughout the plant; the leaves contain two times as much as the stems per unit weight. Therefore, for uniform sampling the plants were oven-dried at 65-70 C, to 4% moisture, ground in a Wiley mill, and subsequently thoroughly mixed. A portion of the plant material was extracted in a soxhlet apparatus for 8 hr, with either heptane or acetone, followed by methanol. Results are shown in Figure 1.

Chemical Characterization of the Heptane Extract

Several chromatographic and spectroscopic methods were used in chemical characterization of this complex mixture. By column and thin layer chromatography the mixture was separated into subfractions, which were amenable to GLC analysis. Structural information was obtained by combined gas chromatography-mass spectroscopy (GC-MS) and elemental composition by high resolution mass spectroscopy (HRMS).

The heptane extract (or the heptane soluble portion of the acetone extract) was first separated by column chromatography on silica gel into five crude fractions by elution with solvents of increasing polarity. Infrared spectra were used to determine functional groups present in each column fraction as shown in Table I. With the exception of column fraction I, all other fractions were either separated further

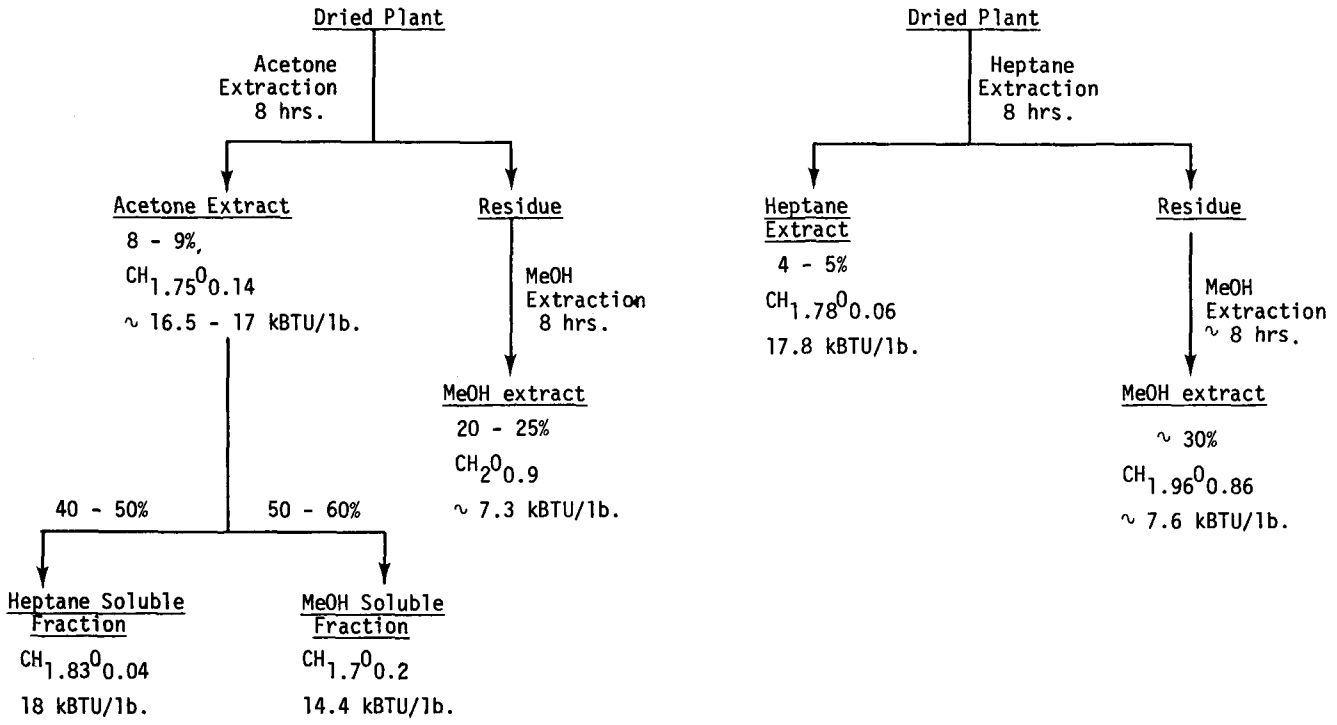


FIG. 1. Alternate extraction methods of *Euphorbia lathyris*.

TABLE I

Heptane Fraction on Silica Gel Column[a]

Eluent		Elemental	Functionality	
I, Heptane	7%	CH_2	---	
II, Benzene	33%	$CH_{1.72}O_{0.03}$	RC–OR O	C = C
III, EtoAc	41%	$CH_{1.67}O_{0.07}$	–OH C=O	C = C
IV, Acetone	5%	$CH_{1.63}O_{0.15}$	–OH C=O	C = C
V, MeOH	14%	$CH_{1.69}O_{0.22}$	–OH C=O	C = C

[a]The silica gel was prewashed with methanol, then reactivated.

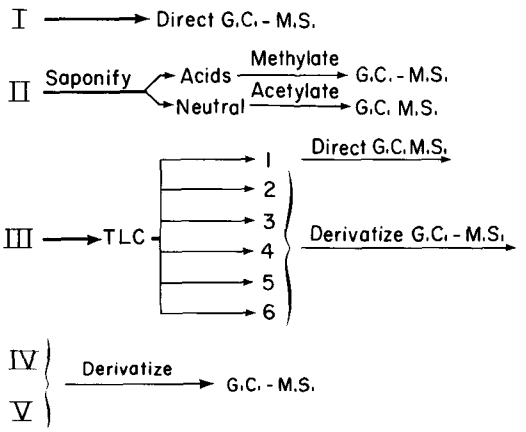


FIG. 2. Analysis of silica gel column fractions.

by thin layer chromatography (TLC), saponified and/or appropriately derivatized prior to GC-MS analysis. Figure 2 outlines the subsequent treatment of each column fraction.

EXPERIMENT

Thin layer separations were done on 500μ thick commercial silica gel plates purchased from Analtech Inc. The eluent for the thin layer separation of column fraction III was petroleum ether/ether (1:1).

GLC analyses were performed on either a 50 m 0.5 mm ID OV-101 or a 37 m 0.5 mm ID SE-30 glass column at 250 or 280 C. Appropriate blanks were run in all cases. GC-MS data were obtained on a Dupont 21-491 MS coupled to a Varian Aerograph GC. High resolution MS data were obtained on a MS 902 or Dupont CEC 21-110B instrument at a resolving power of 10,000. Measured masses were within 5 ppm of calculated values.

Fatty acids were methylated with BF_3 methanol (purchased from Supelco). Acetylations were done with redistilled Ac_2O/pyr (4:1) at 80 C for 3 hr. TMS ethers were prepared by using BFTSA with a drop of pyridine and heating at 70 C for 3 hr.

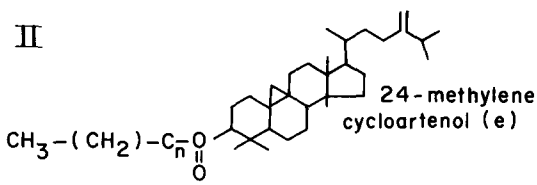
RESULTS AND DISCUSSION

Results of GC-MS analysis of each column and TLC fraction are summarized in Tables II and III. Only major components are shown. Molecular weights are indicated for the parent alcohols where pertinent. Elemental compositions were obtained from HRMS measurements, sometimes on the parent ion of the derivatized sample, but more often on the usually very intense M-60 ion in the case of the acetates, or the M-90, or M-15 ion in the case of the TMS ethers. The relative ratios of the individual components, shown in parentheses, were obtained from the areas of the GLC peaks.

The data indicate that the *Euphorbia lathyris* heptane

TABLE II

Column Fractions I, II, IV, V

		No. of components	M. W.	
I	$n\text{-}C_{31}H_{64}$ $n\text{-}C_{33}H_{68}$	2	a-438 (1) b-464 (0.62)	
II		5	ACIDS n=16,18,20,22--major 28,30--minor a-426 (0.17) b-426 (0.09) c-426 (0.38) d-426 (0.89) e-440 (1)	$C_{30}H_{50}O$ $C_{31}H_{52}O$
IV	bifunctional triterpenoids and sterols	8	a-428 b-428 c-428 d-432 e-458 f-458	$C_{29}H_{48}O_2$ $C_{31}H_{54}O_2$
V	bifunctional triterpenoids and sterols	5	a-428 b-468	$C_{29}H_{48}O_2$ (a)

IIb: lanosterol

IId: cycloartenol

extract is composed almost entirely of polycyclic triterpenoids functionalized as fatty acid esters, ketones, or alcohols. Triterpenoids constitute a large and complex class of natural compounds. However, by comparison of mass spectra and GLC retention times (coinjection) with authentic standards, we were able to make the structural assignments as indicated for some selected components in Tables II and III.

Structural elucidation of some of the other major components was based on mass spectral fragmentation, GLC retention behavior, as well as on elemental composition of diagnostic ions. Numerous detailed, systematic studies of the fragmentation patterns of sterols and triterpenoids have been published (6-8). The reported characteristic mass spectral features were used for skeletal assignment, and for recognition and location of functional groups. For the set of monofunctional compounds, for which standards were not available for comparison of GLC retention times, the pertinent data as well as some very probable structural assignments are shown in Table IV.

The components of fraction III-5 as well as those of Fraction IV and V can be classified at this point into the broad class of bifunctional triterpenoids. The mass spectra of the five isomeric compounds III-5-b-f show successive loss of two molecules of trimethylsilanol and loss of methyl from the intense M-90 and M-180 ions. The base peak in all the spectra is the M-180 ion, from which the reported elemental composition was obtained.

The mass spectra of components IVa, b, and c show fragmentation patterns which can be best attributed to a sterol molecule. None of the mass spectra showed a molecular ion, but the M-60 ion (loss of acetic acid) is the base peak. This ion has an elemental composition of $C_{29}H_{46}O$, indicating that the original compounds have more than one oxygenated function. The fragment ion in the spectrum of V-c which corresponds to loss of a 10 carbon side chain has an elemental composition of $C_{19}H_{25}O$, signifying that both oxygen functionalities are on the sterol nucleus in this case.

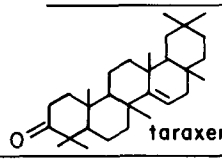
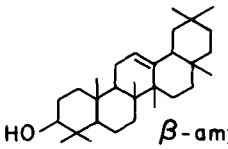
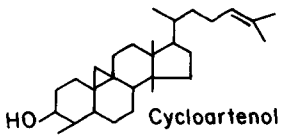
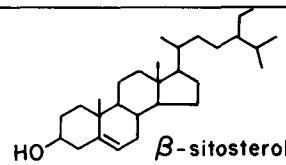
Similarly, the mass spectra of the other components in fraction IV are indicative of tetracyclic triterpenoids, but with a 9 carbon side chain. Component f also exhibits a fragmentation typical of a 9,19 cyclopropyl sterol skeleton. However, the significantly longer GLC retention times of these components (~ 100 retention index units) compared to cyclolaudenol preclude the possibility that these compounds are isomeric with it. The most reasonable interpretation is that these are oxygenated derivatives of C_{31} compounds such as 24-methylene cycloartenol or cyclolaudenol.

The fragmentation patterns of the compounds present in the most polar fraction V are also indicative of sterols 8, 9, and 10 carbon side chains. The GLC retention behavior and mass spectra of the major components are consistent with a C_{29} bifunctional sterol. Structure elucidation work is still continuing for these polar components.

The heptane extract of *Euphorbia lathyris* consists almost exclusively of triterpenoids and sterols. Several of these compounds were previously identified as the major constituents of the latex (9); all of the latex components with the exception of euphol (a minor one) are also present in the whole plant extract. Since *Euphorbia lathyris* cannot

TABLE III

Column Fraction III

		No. of components	M. W.	
①	 taraxerone (a)	2	a-424 (1) b-424 (0.42)	$C_{30}H_{48}O$
②	 β-amyrin (b)	5	a-426 (0.46) b-426 (0.21) c-426 (0.1) d-426 (0.2) e-440 (1)	$C_{30}H_{50}O$ $C_{31}H_{52}O$
③	 Cycloartenol (b)	4	a-426 (0.1) b-426 (0.25) c-426 (0.1) d-440 (1)	$C_{30}H_{50}O$ $C_{31}H_{52}O$
④	 β-sitosterol (b)	3	a-412 (0.3) b-414 (1) c-416 (0.25)	$C_{29}H_{48}O$ $C_{29}H_{50}O$ $C_{29}H_{52}O$
⑤	bifunctional triterpenoids	8	a-424 b-f-442	$C_{30}H_{50}O_2$
⑥	fatty alcohols	3	394 (0.6) 410 (1) 422 (0.4)	$C_{27}H_{54}O$ $C_{28}H_{58}O$ $C_{29}H_{58}O$

III-2-a: taraxerol

III-2-e: 24-methylenecycloartenol

TABLE IV

Component	Structure	MS features
		Acetates
IIa IIc	Isomers of lanosterol	468(M^+), 453(M-15), 408(M-60), 399(M-60-15, b.p.) 365, 339, 301, 297, 295, 255.
III-1 b	Arborenone	424(M^+), 409(M-15), 339, 271, 257 (b.p., $C_{18}H_{25}O$)
		Acetates
III 2-c III-2-d	Lupeol Isomer of lupeol	468 (M^+), 408(M-60), 393(M-60-15), 365, 297, 218, 203 189(b.p.)
		TMS ethers
III-3a III-3-c	Triterpenoid	498(M^+), 483(M-15), 408(M-90), 393(M-105), 365 (M-90-43, $C_{27}H_{41}$), 287, 274($C_{20}H_{34}$), 259($C_{19}H_{31}$), 144(b.p.)
III-3-d	Isomer of cyclolaudenol	512(M^+), 297(M-15), 422(M-90 b.p.), 407(M-105), 379, 353, 339, 300, 297.
		TMS ethers
III-4a	Δ_5 Sterol C_{10} side chain	484(M^+), 469(M-15), 394(M-90), 379(M-105), 355(M-129), 351, 255, 129(b.p.)
III-4c	Stanol, C_{10} side chain	488(M^+), 473(M-15), 398(M-90), 383(M-105), 305, 215(b.p.)

be tapped conveniently, like some other *Euphorbs,* we chose the whole plant extract for study as more representative from a practical viewpoint. The plant extract, however, yields a much greater variety of triterpenoids than the latex. Since no lower terpenoids have been found to date as major constituents of *Euphorbia lathyris* (10), it appears that terpenoid biosynthesis in this plant is shunted almost exclusively via the squalene cyclization route.

While *Euphorbia lathyris* contains a significant amount of isoprenoids in a reduced state, further conversion of this material is desirable, if it is to be used as conventional fuel. The chemical structure of these compounds, however, suggests that conversion to chemical feedstock material might be advantageous. Such conversion studies have already been carried out on vegetable oils and a *Euphorbia* latex (11).

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