SUMMARY

Isopropyl myristate was used as the receptor phase to evaluate the release of fluocinolone acetonide and its acetate ester from gelled propylene glycol–water vehicles. Steroid release was found to be a function of its (*a*) concentration, (*b*) solubility in the vehicles, and (*c*) partition coefficient between vehicles and receptor phase. Release was reduced if a vehicle contained cosolvent in excess of that required to dissolve the steroid. Release could be improved not only for the different steroids, but also for different concentrations of the same steroid, by use of the proper vehicle.

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Keyphrases

Vehicles, topical—steroid release
Propylene glycol–water–Carbopol 934—vehicles
Fluocinolone acetonide and its acetate–test compounds
Radioactive compounds—release rates
Liquid scintillation counting—analysis
Solubility—propylene glycol–water
Partition coefficient—isopropyl myristate–propylene glycol system

Euphorbia esula L. (*Euphorbiaceae*) I

Preliminary Phytochemical and Biological Evaluation

By N. R. FARNSWORTH*, H. WAGNER, L. HÖRHAMMER, H. P. HÖRHAMMER, and H. H. S. FONG

The aerial parts of *Euphorbia esula* were subjected to a fractionation scheme which resulted, after several chromatographic separations, in the isolation of β-sitosterol, 24-methylenecycloartanol, hexacosanol-(1), *n*-nonacosane, and *n*-hentriacontane. The presence of *n*-pentacosane, *n*-hexacosane, *n*-heptacosane, *n*-octacosane, *n*-triacontane, *n*-dotriacontane, and *n*-tritriacontane was demonstrated by means of gas chromatography. None of these compounds had previously been reported as isolated from *Euphorbia esula*. The presence of flavonoids and alkaloids was demonstrated, a finding that is contrary to literature reports. A defatted ethanol extract elicited no antimicrobial activity against several test organisms and was found to induce only weak central nervous system depression in mice. Seven tumor systems were not inhibited by the extract, and it was not cytotoxic in cell culture.

LIMITED phytochemical investigations on *Euphorbia esula* L. have shown only the presence of L-inositol (1), gallic acid (2), rubber (2), and a number of common amino acids (3). Conversely, phytochemical screening of this plant has indicated the absence of hemolytic saponins, flavonoids, alkaloids, and tannins (4). Peroxidase and polyphenoloxidase activity has been reported for rootbud extracts (3), and an inhibitor of indole-3-acetic acid has been shown to be present in whole plant extracts (5).

Folkloric usage for the treatment of cancer has been recorded for several species of *Euphorbia*

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(*E. ingens*, *E. bupleurifolia*, *E. clavarioides*, *E. gorgonis*) (6), and experimentally, extracts of *Euphorbia amygdaloides* (7), *E. drummondii* (8), *E. marginata* (9), *E. pilulifera* (8), and *E. resinifera* (8) have been reported to induce tumor remissions in laboratory animals.

Interest was piqued by the limited phytochemical reports on *Euphorbia esula*, and by the interesting biological activity reported for this and related species of this genus; therefore, an investigation of this plant was initiated.

EXPERIMENTAL

Plant Material—Plant material used in this investigation was collected in Greensburg, Pa., during May and August 1964. Whole, flowering plants were uprooted, air-dried, separated into roots and aerial parts, and milled to a coarse powder. Voucher specimens were prepared and authenticated as *Euphorbia esula* L. (*Euphorbiaceae*).[1] Specimens representing the collections have been deposited in the herbaria of Carnegie Museum, and in the Department of Pharmacognosy, University of Pittsburgh, Pittsburgh, Pa.

Extraction and Fractionation—Only the aerial parts were used in this study. A total of 27.57 Kg. of plant material was extracted continuously with skellysolve B (*n*-hexane) for 72 hr. in a Lloyd extractor. Concentration of this extract *in vacuo* to about 4 L. yielded, on standing overnight, 116 Gm. of a gray powder (Fraction *A*). The mother liquor remaining from the removal of Fraction *A* was reduced to dryness to yield 410 Gm. of residue (Fraction *B*).

The skellysolve B-exhausted plant material was again continuously extracted with methanol for 72 hr. in a Lloyd extractor. This extract was concentrated to about 4 L., 4 L. of distilled water was added, and the mixture concentrated *in vacuo* until all traces of methanol were removed. Lyophilization of the methanol-free mixture yielded 1,688 Gm. of dark-green, dry powder (Fraction *C*) (Scheme I).

Isolation of Hexacosanol-(1) from Fraction A—Thin-layer chromatography of Fraction *A* on Silica Gel G plates, using a developing solvent composed of *n*-hexane–ethyl acetate (75:25), revealed that the fraction consisted mainly of a single spot at R_f 0.56, with small amounts of a substance traveling at or near the solvent front. The detecting reagent was 70% sulfuric acid, applied as a thin spray, followed by heating the treated plates at 120° for 10–15 min.

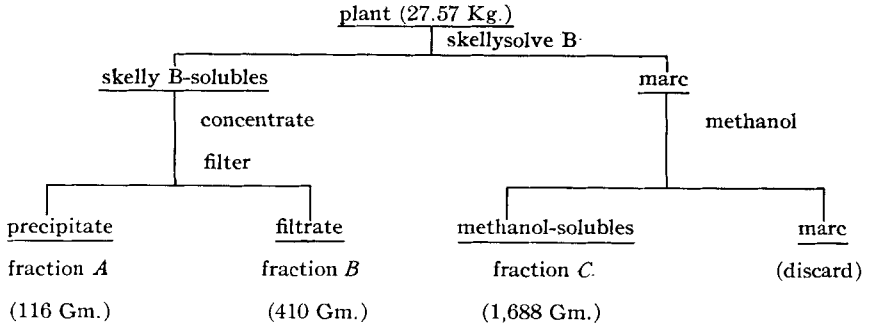
A small amount of Fraction *A* was purified by boiling it with acetone, filtering, and cooling the filtrate to room temperature. An amorphous precipitate formed which was removed, dissolved in hot chloroform, boiled for several minutes with decolorizing charcoal, and the mixture filtered. This filtrate, on standing at room temperature, produced a copious white precipitate which was removed and dried in a desiccator at room temperature *in vacuo* for 48 hr. Thin-layer chromatography of this material, as previously described, indicated a homogeneous one-spot material. The sample was identified as hexacosanol-(1) on the basis of its having melting point 76.5–77° (cap., uncorr.) [lit. m.p. 79.5–79.8° (10)], and a specific rotation $[\alpha]_D^{23}$ + 0° (c 1.0, $CHCl_3$).

Anal.—Calcd. for $C_{26}H_{54}O$: C, 81.60; H, 14.22. Found: C, 81.86; H, 14.00.

An infrared spectrum (KBr) was identical with that of an authentic sample of hexacosanol-(1), and a mixed melting point of the isolated material and the authentic sample was not depressed.

Saponification and Separation of Fraction B—An aliquot (195 Gm.) of Fraction *B* was dissolved in about 1 L. of 95% ethanol, 30 Gm. of KOH added, and the mixture refluxed for 1.5 hr. Removal of the ethanol was accomplished by means of a flash evaporator and the residue remaining was mixed with 1 L. of distilled water. This mixture was then extracted several times with petroleum ether (b.p. 30–60°), the combined petroleum ether extracts (about 3 L.) washed with distilled water, and the aqueous phase discarded. Attempts to reduce the petroleum ether extract to dryness, *in vacuo*, yielded a thick oil. This oil was mixed with about 1 L. of acetone, heated on a steam bath, and filtered while hot to remove 11 Gm. of a black tar (Fraction *B*-1). Cooling the filtrate to room temperature resulted in the deposition of a gray, amorphous precipitate, which was removed by filtration, air-dried, and labeled Fraction *B*-2 (4 Gm.). The Fraction *B*-2 mother liquor was concentrated to about 500 ml. and formed, on standing at room temperature, a copious precipitate which weighed 30 Gm. (Fraction *B*-3). Concentration of the mother liquor remaining from the isolation of

[1] Dr. Leroy K. Henry, Carnegie Museum Herbarium, Pittsburgh, Pa.



Fractionation Scheme for Euphorbia esula aerial parts
Scheme I

Fraction *B*-3, to about 250 ml. yielded 27 Gm. of an additional precipitate (Fraction *B*-4). This mother liquor was reduced to dryness, about 1 L. of *n*-hexane was added, the mixture heated to boiling, and then filtered while still hot. Concentration of the filtrate (*n*-hexane-solubles) to dryness yielded 32 Gm. of Fraction *B*-5. The filtration residue (*n*-hexane-insoluble material), which represented a considerable weight of material, was mixed with about 1 L. of acetone, the mixture heated to boiling, and filtered while hot. Evaporation of the filtrate to dryness yielded 55 Gm. of acetone soluble material (Fraction *B*-6). The acetone-insoluble residue (Fraction *B*-7). weighed 3 Gm.

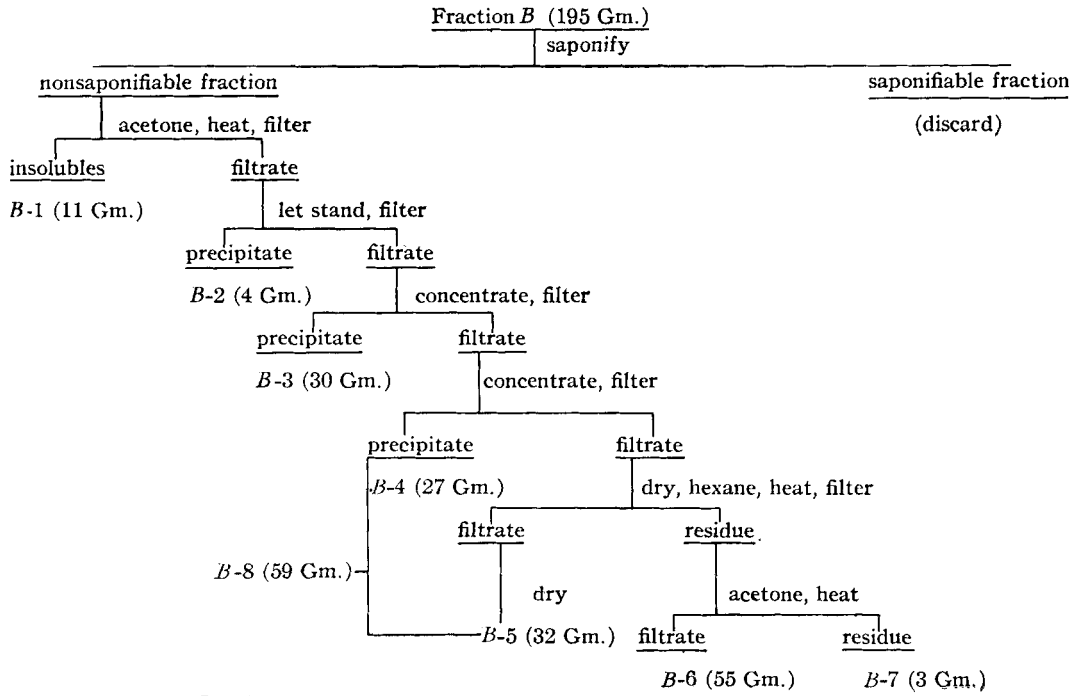
Thin-layer chromatography of Fractions *B*-1 to *B*-7 was accomplished on silica gel G plates, utilizing a solvent system of *n*-heptane–benzene–ethanol (50:50:0.5), and revelation of spots was effected by spraying each plate with 70% sulfuric acid, followed by heating at 120° for 15 min. Fractions *B*-1, *B*-2, and *B*-3 were found to exhibit identical chromatographic patterns. Each exhibited a major spot at R_f 0.11 and trace amounts of *n*-alkanes migrating at or near the solvent front. The R_f 0.11 spot in each fraction corresponded to hexacosanol-(1) in this and in several other solvent systems; therefore, no further studies were made on these fractions.

Fractions *B*-4 and *B*-5, however, showed the presence of several spots, giving characteristic color reactions suggestive of the presence of triterpenes and/or sterols (11), in addition to hexacosanol-(1) and *n*-alkanes. Since these two fractions were almost identical in migration patterns and chromogenic response of the resolved components to the spray reagent, they were combined and marked as Fraction *B*-8 (59 Gm.). Fractions *B*-6 and *B*-7 exhibited dissimilar chromatographic patterns and therefore were not combined (Scheme II).

Investigation of Fraction B-8—The fraction (59 Gm.) was dissolved in a minimum volume of hot petroleum ether (b.p. 30–60°) and this solution held overnight at room temperature. Crystals which formed were removed and recrystallized from petroleum ether in a similar manner yielding 0.194 Gm. of material having a melting point of 75–77° (cap., uncorr.). Comparison of this isolate with hexacosanol-(1) by means of mixture melting point, optical rotation, infrared absorption spectrum, and thin-layer chromatography, showed them to be identical.

After removal of the hexacosanol-(1), the fraction was taken to dryness, dissolved in a minimum volume of *n*-hexane, and added to the top of a column (5.5 × 55 cm.) of Woelm, neutral, activity grade I alumina, packed as an *n*-hexane slurry. Elution was initiated with *n*-hexane, followed by *n*-hexane–benzene (1:1), benzene, ether, and methanol. One hundred-milliliter fractions were collected, reduced to about 10 ml., and subjected to thin-layer chromatography as previously described, using a solvent system of *n*-heptane–benzene–ethanol (50:50:0.5). Fractions were grouped according to the eluting solvent used, taken to dryness, and crystallizing attempts initiated with the usual solvents. These data are presented in Table I.

Isolation of *n*-Nonacosane and *n*-Hentriacontane—Fractions 1–41 were combined, taken to dryness, and dissolved in a minimum volume of *n*-hexane. Chilling produced a material which deposited as shiny plates when dried *in vacuo* at room temperature for 24 hr. This isolate was weighed (0.685 Gm.) and found to have a m.p. of 67–68° (cap., uncorr.). The sample exhibited no ultraviolet absorption spectrum and had optical rotation $[\alpha]_D^{24} \pm 0°$ (c, 1.25, $CHCl_3$). An infrared absorption spectrum (KBr) showed typical absorption peaks suggestive only of *n*-alkanes, with an absence of OH absorption. The



Fractionation Scheme for Euphorbia esula Aerial Parts, Fraction B
Scheme II

TABLE I—CHROMATOGRAPHIC SEPARATION OF FRACTION *B*-8[a]

Fraction,[b] No.	Eluent	Isolated Compd.	Yield, Gm.	Crystn. Solvent
1–41	*n*-Hexane	*n*-Alkane mixture	0.685	*n*-Hexane
42–57	*n*-Hexane–benzene (1:1)	...	...	...
58–152	Benzene	Hexacosanol-(1)	0.331	Benzene
153–193	Ether	Hexacosanol-(1)	0.031	Methanol
194–210	Methanol	...	...	...

[a] On Woelm, neutral, activity grade I alumina. [b] Each fraction was 100 ml.

sample was analyzed by gas chromatography, using the conditions described in an earlier work (12), and was found to be composed of the following *n*-alkanes: *n*-pentacosane (<1%), *n*-hexacosane (<1.0%), *n*-heptacosane (<1%), *n*-octacosane (<1%), *n*-nonacosane (31%), *n*-triacontane (2%), *n*-hentriacontane (53%), *n*-dotriacontane (2%), and *n*-tritriacontane (7%). An additional sample of this *n*-alkane mixture was subjected to a preparative gas chromatographic separation and the major peaks, corresponding to the C_{29} and C_{31} *n*-alkanes, were collected. Melting point determinations (cap., uncorr.) on the two isolates showed m.p. 63.0–63.5° for the C_{29} peak isolate, and 67.5–68.0° for the C_{31} peak isolate, which corresponded to m.p. 63.4–63.7° and 67.5–68.5° reported in the literature for *n*-nonacosane and *n*-hentriacontane, respectively (10).

Direct crystallization procedures performed on the other column chromatographic fractions yielded only hexacosanol-(1) (Table I).

Separation of Fraction B-8 (58-152)—After removal of the hexacosanol-(1), obtained by direct crystallization procedures, the material eluted by benzene during the column chromatography of Fraction *B*-8 (column Fractions 58–152) was dried, dissolved in a minimum volume of benzene, and rechromatographed on this column with benzene as the initial eluent. Again, 100-ml. fractions were collected, each fraction concentrated to about 10 ml., and monitored by means of TLC using *n*-heptane–benzene–ethanol (50:50:0.5) as previously described. Based on changes in the TLC patterns, eluents were increased in polarity and fractions were pooled as indicated in Table II. Combined Fractions 69–83 were taken to dryness, dissolved in hot methanol, and the resulting solution chilled. After 24 hr. crystals formed which, after they were harvested and dried *in vacuo* in an Abderholden pistol over boiling methanol for 24 hr., weighed 0.247 Gm., and melted at 112–113.5° (Kofler, uncorr.). Thin-layer chromatography of the sample, as previously described, utilizing *n*-heptane–benzene–ethanol (50:50:2.0) as the developing solvent, showed it to be a homogeneous one-spot material, exhibiting an R_f of 0.19, which was identical to the R_f of a reference sample of 24-methylenecycloartanol.[2]

Identification of 24-Methylenecycloartanol—The isolate having m.p. 112–113.5° [lit. m.p. 118–119° (13)] exhibited an optical rotation of $[\alpha]_D^{24}$ + 47.9° (c 1.087, $CHCl_3$) [lit. $[\alpha]_D^{14.5}$ + 48.8° (c 0.26, $CHCl_3$) (13)], and an infrared absorption spectrum (KBr) that was identical with that of reference 24-methylenecycloartanol[2] (Fig. 1).

Anal.—Calcd. for $C_{31}H_{52}O$: C, 84.48; H, 11.89. Found: C, 84.03; H, 11.86.

Acetylation of the isolate was accomplished with acetic anhydride in pyridine in the usual manner, and the acetate crystallized from ethanol to give needles having m.p. 105–106° [lit. m.p. 110–111° (13)], and optical rotation $[\alpha]_D^{24}$ + 56.8° (c 1.467, $CHCl_3$), lit. $[\alpha]_D^{18.5}$ + 56.6° (c 0.49, $CHCl_3$) (13).

Anal.—Calcd. for $C_{33}H_{54}O_2$: C, 82.10; H, 11.27; $OCOCH_3$, 10.96. Found: C, 81.60, 81.68; H, 11.20, 11.42; $OCOCH_3$, 10.95.

Isolation of β-Sitosterol—Fractions 84-128 (12.7 Gm.) (Table II) from the column chromatographic separation of the combined Fractions 58–152 obtained from the column chromatography of Fraction *B*-8 would not yield crystals on direct crystallization attempts from the usual solvents. Therefore, 9.5 Gm. of the fraction was dried, dissolved in 40 ml. of *n*-hexane, applied to the top of a glass chromatographic column (5.5 × 49 cm.) containing an *n*-hexane slurry of silicic acid (Kieselgel-Merck, 0.05–0.20 mm.), which had previously been activated by

TABLE II—COLUMN CHROMATOGRAPHIC SEPARATION OF FRACTION *B*-8(58–152)(58 Gm.)[a]

Fraction,[b] No.	Eluent	Combined Fraction No.	Fraction Wt., Gm.	Isolated Compd.	Yield, Gm.
1–33	Benzene	1–38	1.3	...	...
34–96	Benzene–ether (9:1)	39–68	6.8	...	...
	Benzene–ether (9:1)	69–83	6.7	24-Methylenecycloartanol	0.247
	Benzene–ether (9:1)	84–128	12.7	...	...
97–113	Benzene–ether (8:2)	...			
114–125	Benzene–ether (1:1)	...			
126–136	Methanol	129–136	1.8	...	...

[a] On Woelm, neutral, activity grade I alumina. [b] Each fraction was 100 ml.

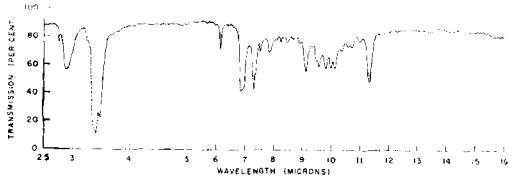


Fig. 1—Infrared absorption spectrum of 24-methylenecycloartanol (KBr).

[2] Reference samples of 24-methylenecycloartanol were supplied through the courtesy of Drs. Wesley Cocker, A. N. Starratt, and G. Ponsinet.

heating at 110° for 24 hr., and elution was initiated with *n*-hexane. Fractions (100 ml.) were collected, grouped on the basis of TLC behavior as previously described, and on the basis of shifts in TLC patterns, eluents were changed as indicated in Table III. Fractions 17–22 were combined, taken to dryness, and the residue dissolved in a minimum volume of hot methanol. A small amount of decolorizing charcoal was added, the mixture boiled for a few minutes, filtered until clear, and refrigerated for 24 hr. Crystals that formed were removed and, after drying *in vacuo* over boiling methanol for 24 hr., were shown to be one-spot material (R_f 0.09) on TLC as previously described, using the solvent system *n*-heptane–benzene–ethanol (50:50:2.0). This isolate was found identical in its chromatographic behavior, in several solvent systems, with β-sitosterol. The yield was 0.319 Gm. and the crystals had m.p. 133–135° [lit. m.p. for β-sitosterol 137–138° (14, 15)]. Optical rotation $[\alpha]_D^{22}$ −22.58° (c 0.80, $CHCl_3$), lit. $[\alpha]_D$ −37.8° (c 1.2, $CHCl_3$) (16).

Anal.—Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 84.02, 83.92; H, 12.14, 11.75.

The infrared absorption spectrum was identical in all respects with that of a reference sample of β-sitosterol.[3] Acetylation of a small sample of the isolated product with acetic anhydride in pyridine in the usual manner produced an acetate which, after crystallization from ethanol, gave needles having m.p. 124–125° [lit. m.p. for β-sitosterol acetate 126–128° (17)].

Anal.—Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48; $OCOCH_3$, 11.48. Found: C, 81.25, 81.14; H, 11.28, 11.36; $OCOCH_3$, 10.62.

An infrared spectrum of this acetate was identical with that of an acetate prepared as described above from authentic β-sitosterol.

Fractions 23–26 yielded 0.125 Gm. of crystals from chloroform–methanol that were identical in all respects with the β-sitosterol isolated from Fractions 17–22.

Treatment of Fractions 11–14, in methanol, with decolorizing charcoal, yielded 0.065 Gm. of crystals that were identical with the 24-methylenecycloartanol previously isolated from Fractions 69–83 from the column chromatographic separation of Fraction *B*-8 (58–152). Crystals could not be induced to form from the remaining fractions, either before or after acetylation.

Separation of Flavonoids—Fraction C (500 Gm.) was dissolved in about 2 L. of distilled water with the aid of heat, the resulting solution concentrated on a steam bath to about 1 L., and then refrigerated for 24 hr. During this time a green scum separated, which was removed by filtration, to give a clear brown solution. This solution was extracted several times with chloroform, which on drying yielded Fraction *C*-1 (6.78 Gm.). The aqueous phase remaining from the chloroform extraction was then extracted several times with diethyl ether, which, following evaporation of the ether and drying, yielded 2.97 Gm. of Fraction *C*-2. Fractions *C*-3 (19.50 Gm.) was obtained by extracting the aqueous phase several times with ethyl acetate. Finally, the aqueous phase was extracted several times with ethyl acetate–methanol (9:1) to yield 5.93 Gm. of Fraction *C*-4. Thin-layer chromatography of Fractions *C*-1, *C*-2, *C*-3, and *C*-4 on polyamide plates, using methanol as the developing solvent, and followed by spraying each plate with an aqueous saturated solution of lead subacetate, revealed the presence of several fluorescent and visible spots in each fraction that were suggestive of the presence of flavonoids (18). Work is now in progress toward the isolation and identification of the flavonoid constituents in each of these fractions.

Detection of Alkaloids—A 20-Gm. sample of Fraction *C* was dissolved in methanol, mixed with 250 ml. of 5% hydrochloric acid, and the mixture was steam distilled *in vacuo* to remove the methanol and convert any alkaloids present to hydrochloride salts. This mixture was then filtered, and the filtrate was made basic to litmus with 28% ammonium hydroxide solution. Three 500-ml. and six 250-ml. chloroform extractions of the basic solution were pooled, dried over anhydrous sodium sulfate, and filtered. This filtrate was then taken to dryness to yield 300 mg. of residue, which was designated as the tertiary alkaloid fraction.

The alkaline mother liquor was acidified with 1.0 *N* hydrochloric acid, refrigerated overnight, and then filtered. This solution was next concentrated by the addition of methanol and evaporation *in vacuo* using a flash evaporator. During the concentration procedure, copious amounts of a white crystalline material separated (about 18 Gm. was collected), which was shown to be ammonium chloride. The extract was then taken to dryness, treated with hot acetone, and filtered to remove the quaternary bases. This treatment failed to remove a large quantity of residual crystalline material, which was presumed to be ammonium chloride. The acetone solution was designated as the quaternary alkaloid fraction.

TABLE III—COLUMN CHROMATOGRAPHIC SEPARATION OF FRACTIONS 84–128 (9.5 Gm.)[a]

Fraction,[b] No.	Eluent	Fraction Wt., Gm.	Isolated Compd.	Yield, Gm.
1–3	*n*-Hexane	0.15	...	...
4–10	*n*-Hexane–benzene (9:1)	0.20	...	...
11–14	*n*-Hexane–benzene (1:1)	1.10	24-Methylenecycloartanol	0.065
15–16	Benzene	5.01	...	...
17–22	Benzene–ether (9:1)	2.80	β-Sitosterol	0.319
23–26	Ether	0.30	β-Sitosterol	0.125
27–32	Methanol	0.10	...	...

[a] On silicic acid (Kieselgel-Merck, activated, 0.05–0.20 mm.). [b] Each fraction was 200 ml.

The tertiary alkaloid fraction (300 mg.) was dissolved in 15 ml. of chloroform. Ten milliliters of this solution was mixed with 10 ml. of 5% hydrochloric acid solution, the mixture was shaken, and the acidic portions removed. Two-milliliter aliquots of this acidic extract were placed in each of four test tubes, and three drops of the following reagents were added

[3] A reference sample of β-sitosterol was supplied by Dr. G. H. Constantine, Jr.

TABLE IV—THIN-LAYER CHROMATOGRAPHY OF *E. esula* ALKALOID FRACTIONS[a]

Solvent System	R_f Values of Resolved Alkaloids: Tertiary Fraction	Quaternary Fraction	Control[b]
n-Propanol–NH_4OH–water (2:1:1)	0.87	0.95, 0.88, 0.73	0.88
Ethyl methyl ketone–methanol–10% NH_4OH (6:3:1)	0.27, 0.17	0.85, 0.15	0.22
Methanol–NH_4OH(99:1)	0.85, 0.05	...	0.25

[a] On Silica Gel G plates (250 μ). [b] *l*-Hyoscyamine.

separately to the contents of each tube: Valser's reagent, Mayer's reagent, phosphotungstic acid, Bouchardat's reagent. A moderate precipitate was observed in each of the four tubes after addition of the reagents, indicative of the presence of tertiary alkaloids.

Twenty milliliters of 5% hydrochloric acid solution was added to about one-half of the quaternary alkaloid fraction in acetone, and the acetone removed by means of a flash evaporator. The resulting solution was divided equally between four test tubes and the alkaloid test reagents indicated above were added. A moderate precipitate was observed in each of the tubes, indicative of the presence of quaternary alkaloids.

In order to confirm these initial qualitative tests for alkaloids, both the tertiary and quaternary alkaloid fractions were subjected to thin-layer chromatography on Silica Gel G plates (250 μ) and several different solvent systems.

Because of the reported presence of tropane alkaloids in *Euphorbia* species (19), hyoscyamine was used as a control. The dried chromatograms were sprayed with modified Dragendorff's reagent as a means of detecting the alkaloid components. These results are presented in Table IV.

Biological Activity Screening—A defatted ethanol extract was prepared from *Euphorbia esula* aerial parts as previously described (20). This extract was evaluated for antitumor, antibiotic, and mouse behavior activity, using the protocols described in an earlier work (20).[4] The extract tested by these methods had no antitumor activity at the doses indicated in each of the following tumor systems: adenocarcinoma 755 (30 mg./Kg.), mecca lymphosarcoma (30 mg./Kg.), myeloma X-5563 (30 mg./Kg.), C-1498 leukemia (30 mg./Kg.), and the P-1534 leukemia (7.5 and 15 mg./Kg.). Using different tumors and methods (21),[5] the extract was inactive against the P-1798 lymphosarcoma (31.3–250 mg./Kg.), the Lewis lung carcinoma (31.3–250 mg./Kg.), and the sarcoma 180 (250 mg./Kg.), and had an $ED_{50} > 1.0 \times 10^2$ mcg./ml. (inactive) against Eagle's 9 KB human epidermoid carcinoma of the nasopharynx in cell culture.

The extract was devoid of antibiotic activity against the bacterial and fungal pathogens and saprophytes, the plant pathogens and material-degrading organisms, and the algae and protozoa employed as test organisms in the evaluation (20).[4] At doses ranging from 5–400 mg./Kg., i.p., the extract produced only a weak central nervous system depressant effect in mice (20).[4]

[4] Extracts were evaluated through the courtesy of Eli Lilly and Company, Indianapolis, Ind.

[5] Extracts were evaluated for antitumor activity through the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health, Bethesda, MD 20014. The procedures were those described in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).

DISCUSSION

Whole plant extracts of *Euphorbia esula* L. were previously reported to be devoid of hemolytic saponins, flavonoids, alkaloids, and tannins (4). However, this investigation has shown that several flavonoids are present in the aerial parts of this plant, and at least two tertiary and three quaternary alkaloids have been shown to be present by means of thin-layer chromatography. It is interesting to note that at least 50 species of the genus *Euphorbia* have been tested for the presence of alkaloids, and 25 of them have been recorded as alkaloid-positive. However, it has only been recently that Hart *et al.* succeeded in isolating the first crystalline alkaloid, (+)-9-aza-1-methylbicyclo[3.3.1]nonan-3-one, from the genus *Euphorbia* (*E. atoto*) (19).

Details for the extraction and fractionation of *E. esula* aerial parts have been presented, together with the first report on the isolation of 24-methylenecycloartanol, β-sitosterol, hexacosanol-(1), *n*-nonacosane, and *n*-hentriacontane from this plant.

Hexacosanol-(1) was previously reported isolated from *E. pilulifera* (22), and *E. cyparissias* (14, 23), but not from closely related *E. esula* or any other *Euphorbia* species. The *n*-alkanol, octocosanol-(1), was reported isolated from *E. jolkini* (24) and from *E. regis-jubae* (25); tetracosanol-(1) from *E. watanabei* (15); triacontanol-(1) from *E. hirta* (26), and docosanol-(1) from *E. calculata* (27), but none of these later *n*-alkanols were encountered in the present investigation.

β-Sitosterol has been isolated for the first time from *E. esula*, but it is known to occur in *E. adenochlora* (16), *E. cyparissias* (14), *E. hirta* (26), *E. hirta* var. *procumbens* (28), *E. lathyris* (29), *E. maculata* (25), *E. watanabei* (15), and *E. pulcherrima* (30).

The tetracyclic triterpene, 24-methylenecycloartanol, which is a probable intermediate in the biosynthesis of phytosterols (31), was also isolated for the first time from *E. esula*, as the major constituent of the triterpene fraction. Its presence in other *Euphorbia* species appears to be restricted to *E. cyparissias* (14). On the basis of interpretation of thin-layer chromatograms of the several *E. esula* nonsaponifiable fractions separated in this investigation, it is evident that several triterpenes are present in this plant. However, all attempts to crystallize them from several column chromatographic separations, either before or after acetylation, were unsuccessful.

The finding of the *n*-alkanes having composition C_{25} to C_{33} in *E. esula*, with *n*-nonacosane and *n*-hentriacontane being present in the highest concentrations, is consistent with the studies of Eglinton *et al.* (32) who investigated the *n*-alkanes of *E. peplus*, *E. balsamifera*, *E. atropurpura*, *E. regis-jubae*, *E. aphylla*, and *E. bourgaeana*, and studied their distribution in this and in other genera.

To date, *n*-nonacosane has been reported isolated from *E. adenochlora* (16) and *E. watanabei* (15, 33); *n*-triacontane from *E. pilulifera* (34), and *E. antisiphilitica* (34); *n*-hentriacontane from *E. pilulifera* (22), *E. lathyris* (29, 33), *E. antisiphilitica* (35), *E. candelilla* var. *luxurians* (36), *E. hirta* (26), *E. hirta* var. *procumbens* (28), *E. humifosa* (22) and *E. thymifolia* (35), and *n*-dotriacontane from *E. antisiphilitica* (35). However, these identifications must all be considered as equivocal, since it has been shown that most reports on the isolation of *n*-alkanes from plants have been erroneous (37, 38). Indeed, in most cases an investigator bases his identity of an *n*-alkane almost completely on melting point, elemental analysis and/or solubility characteristics. It has been shown recently that the substance reported as *n*-nonacosane from *E. watanabei* (15) was, in effect, a complex *n*-alkane mixture having *n*-hentriacontane as the major component (33). Similarly, the *n*-hentriacontane isolated from *E. lathyris* (29) was shown to be a mixture containing only about 53% of this compound, together with varying amounts of six other *n*-alkanes (33).

SUMMARY

(*a*) β-Sitosterol, 24-methylenecycloartanol, hexacosanol-(1) (ceryl alcohol), *n*-nonacosane, and *n*-hentriacontane have been isolated and are reported for the first time from the aerial parts of *Euphorbia esula*.

(*b*) The *n*-alkanes having constitution C_{25} to C_{33} were shown by means of gas chromatography.

(*c*) In addition to flavonoids, small amounts of tertiary, as well as quaternary alkaloids, were shown to be present in the aerial parts of *E. esula*.

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Keyphrases

Euphorbia esula
Phytochemistry—aerial parts, *Euphorbia esula*
Pharmacology—ethanol extract, *Euphorbia esula*
Column chromatography—separation
TLC—separation, identity
Optical rotation—identity
GLC—identity
IR—structure