TABLE II

PHYSICAL PROPERTIES OF DECAETHYLCYCLOPENTASILOXANE

Melting point, °C.	38–39
Boiling point, °C.	167.5 at 1 mm.
$n^{20}D$ (solid)	1.4408
Density, g./ml.	0.968 at 20°
MR_D (obsd.)	139.3
(calcd.)	139.5[5]

The authors determined the dipole moment of this siloxane in benzene solution at 20°. Dielectric constant measurements were carried out at 1,000 cycles using a Schering bridge.[6,7] The observed data are listed in Table III.

TABLE III

DATA FOR THE EVALUATION OF THE DIPOLE MOMENT OF DECAETHYLCYCLOPENTASILOXANE AT 20° IN BENZENE SOLUTION

Mole fraction of solute C_2	Dielectric constant ϵ	Density d	Total molar polarization of solution $P_{1,2}$ cm.³	Total molar polarization of solute P_2, cm.³
0.0871	2.29 ± 0.02	0.8819	39.53	173.5
.0651	2.29	.8809	36.32	173.9
.0370	2.28	.8803	32.06	170.5
.0277	2.28	.8781	30.68	168.9
.0194	2.28	.8779	29.46	166.9
			$P_{2\infty}$ =	162

If 5% of the molar refraction is allowed for the atomic polarization, the dipole moment of the cyclic pentamer (calculated according to the method of Smyth[8]) is found to be 0.88 ± 0.25 *D*. It is interesting that this value is somewhat smaller than the dipole moment of the corresponding methyl compound, 1.35.[9]

Attempts were made to isolate the cyclic hexamer compound following the separation of the trimer, tetramer and pentamer compounds from the mixture of diethyl siloxanes obtained by the hydrolysis of diethyldichlorosilane, but when the distillation pot temperature was raised above that necessary for the separation of the pentamer, the higher diethylsiloxanes were cracked thermally and only the cyclic trimer and tetramer could be collected. Since the thermal rearrangement process in organopolysiloxanes is very sensitive to, and catalyzed by, traces of acid or base, it may be possible to isolate the cyclic hexamer and higher compounds if a rigorous purification of the mixed diethylsiloxanes is made prior to the actual distillation.

We are grateful to Dr. F. E. Luborsky of the Research Laboratory for his measurements of dielectric constants and to Dr. John D. Hoffman for his assistance and helpful suggestions.

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Podophyllotoxin from *Juniperus* Species; Savinin

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In the course of a survey of the conifers for possible damaging effect against Sarcoma 37 in mice, it was found[1] that aqueous suspensions of the dried needles of certain junipers caused hemorrhage and necrosis in this tumor. A search for the components responsible for this activity was instituted, using successive fractionation with different solvents and chromatography, and following the distribution of biological activity in the fractions by quantitative bioassay with mice bearing Sarcoma 37.[2] In this way crystalline podophyllotoxin (one of the tumor-damaging components of podophyllin) was isolated from all the junipers listed (Table I), and from one of these (savin) a new substance called savinin, inactive toward tumors, was also obtained.

The occurrence of podophyllotoxin reported here is interesting in being the first report of this substance in anything but a plant of the *Podophyllum* genus.

Experimental[3]

Fractionation of Savin.—Since savin was the product on which most of the present work was done, the fractionation finally adopted for this material is given. The other species of juniper were treated in similar fashion, and gave a similar distribution of activity. The successive fractions were extracted at room temperature with the different solvents by shaking a few minutes in stoppered flasks until further treatment failed to remove additional material, and the solvents were evaporated off at room temperature; in cases where the extract was gummy, further extraction was facilitated by breaking up the gum with a stirring rod or triturating it in a mortar with the new solvent.

Extraction of the dried ground needles with acetone yielded 17% of a soluble fraction containing essentially all the biological activity. Ligroin treatment of the acetone-soluble fraction removed an inactive soluble part, leaving 47% insoluble containing practically all the activity. Chromatography on activated alumina of an absolute ethanol solution of the ligroin-insoluble fraction, and successive elution with absolute ethanol, 70% ethanol and water gave correspondingly, three cuts from the filtrate. These represented 38, 31 and 11%, respectively, of the original fraction chromatographed, while an estimated 20% remained on the tower. Essentially all the biological activity appeared in the first cut. Treatment of the first cut with ethyl acetate removed 9% of an insoluble, inactive fraction. Extraction of the soluble part with xylene gave 92% of a soluble fraction containing most of the activity. Fractional evaporation of the xylene solution gave a succession of several crops of crystals called A, B, C, D, E and F in yields (based on the original savin) of 0.145, 0.015, 0.043, 0.090, 0.013 and 0.013%, respectively. Fractions A, B and C had similar melting points and high biological activity, while fractions D, E and F had similar melting points and low biological activity. The mother liquor, after separation of the crystals, still had biological activity.

Podophyllotoxin.—Fractions A, B and C were combined (0.20% yield) and recrystallized from benzene yielding colorless needles, m.p. 111° (softens 107°) and having a second m.p. of 185°, $[\alpha]^{21}D$ −123° (*c* 1.03, chloroform). This was identified as podophyllotoxin (m. p. of anhydrous form 183.4°, $[\alpha]^{20}D$ and anhydrous form −132°[4]). Acetyla-

(1) D. B. Fitzgerald, M. Belkin, M. D. Felix and M. K. Carroll, to be published in *J. Natl. Cancer Inst.*, **13**, Feb. (1953).
(2) J. Leiter, V. Downing, J. L. Hartwell and M. J. Shear, *ibid.*, **10**, 1273 (1950).
(3) Melting points in this paper were taken on the Hershberg apparatus and are corrected values.
(4) J. L. Hartwell and A. W. Schrecker, THIS JOURNAL, **73**, 2909 (1951).

tion with acetic anhydride gave the acetyl derivative, m.p. 207–210°, no depression on mixed melting point with an authentic specimen of acetylpodophyllotoxin. Treatment with piperidine in boiling alcohol (footnote 4, procedure for epipicropodophyllin) gave picropodophyllin (m.p. 213–223°) in 75% yield; acetyl derivative, m.p. 211–214°, no mixed m.p. depression with an authentic specimen of acetylpicropodophyllin. The ultraviolet and infrared absorption spectra were identical with those of podophyllotoxin from podophyllin.[5]

Savinin.—Fractions D, E and F were combined (0.12% yield) and crystallized first from benzene then from absolute ethanol, yielding large, colorless, transparent prisms m.p. 146.4–148.4°, $[\alpha]^{21}D$ −87° (*c* 0.95, chloroform).

Anal. Calcd. for $C_{20}H_{14(16)}O_6$: C, 68.57 (68.16); H, 4.03 (4.57); mol. wt., 350.3 (352.3). Found: C, 68.63; H, 4.65; OCH_3, nil.; loss of weight on drying, nil.; mol. wt. (Signer, isopiestic), 360.

The Gaebel test[6] for the methylenedioxy group was positive. The ultraviolet absorption spectrum (Fig. 2) bore no resemblance to that of podophyllotoxin. In the infrared (Fig. 1), the absence of a band around 3450 cm.$^{-1}$ indicates the absence of a hydroxyl group, while bands at 1751 and 1651 cm.$^{-1}$ are strong evidence for the presence of an unsaturated lactone. Savinin was insoluble in cold and hot dilute aqueous sodium hydroxide and therefore has no phenolic or carboxylic acid function. Solubility in alcoholic alkali after short boiling, with formation of a water-soluble salt, indicated a lactone. Structural studies are in progress on this compound.

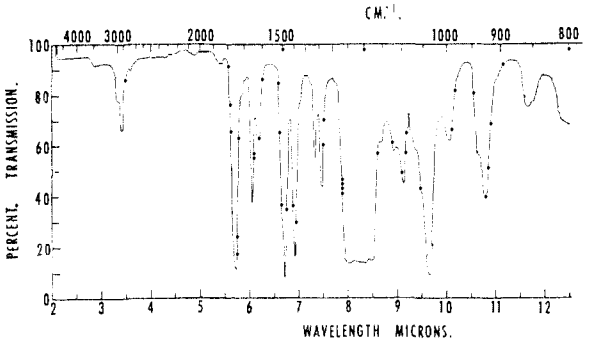

Fig. 1.—Infrared absorption spectrum of savinin in chloroform.

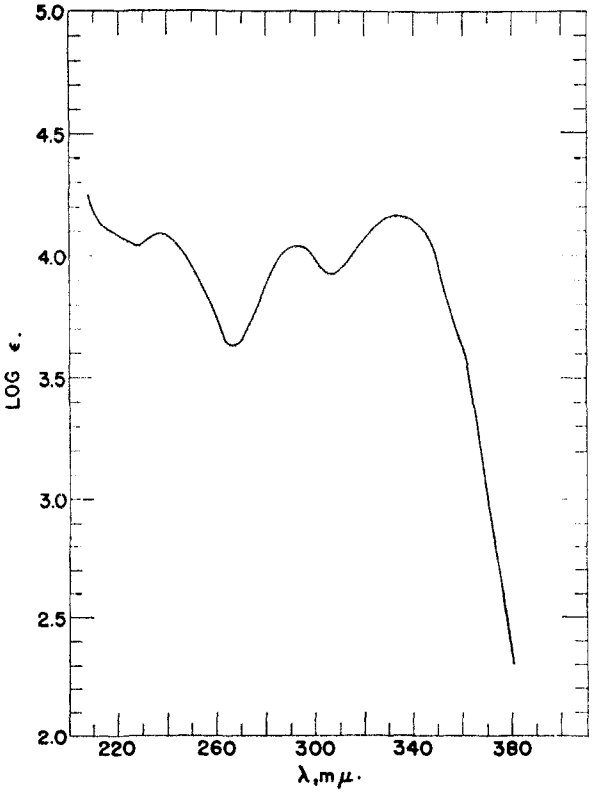

Fig. 2.—Ultraviolet absorption spectrum of savinin in 95% ethanol (based on mol. wt., 298.3).

(5) J. L. Hartwell and W. E. Detty, THIS JOURNAL, **72**, 246 (1950); A. W. Schrecker and J. L. Hartwell, *ibid.*, **74**, 5672 (1952).

(6) G. O. Gaebel, *Arch. Pharm.*, **248**, 225 (1910).

TABLE I

PODOPHYLLOTOXIN IN DIFFERENT *Juniperus* SPECIES

Juniper	Source	Podophyllotoxin, %
Savin[a]	S. B. Penick and Co.	0.20
J. virginiana[b]	Bethesda, Maryland	.10
J. lucayana[c]	Florida	.10
J. scopulorum[d]	California	.17
J. sabina var. *tamariscifolia*, male plant[d,e]	California	.14

[a] The dried needles of an evergreen stated by the supplier to be *J. sabina*. [b] Identified by Mr. E. C. Leonard, Smithsonian Institution, Washington, D.C. [c] Provided through the courtesy of Mr. R. A. Bonninghausen, Florida Board of Forestry, Tallahassee, Fla. [d] Provided through the courtesy of Dr. J. W. Duffield, Institute of Forest Genetics, Placerville, California. [e] The female plant, collected from the same source, yielded a new active substance instead of podophyllotoxin. These results will be reported in a later communication.

Distribution of Biological Activity in Savin.—This is summarized in Table II. The xylene mother liquor is being investigated for other active components.[7]

TABLE II

DISTRIBUTION OF TUMOR-DAMAGING ACTIVITY IN SAVIN

Substance	Yield, %	MED (approx.),[a] μg./g.	Proportion of total activity,[b] %
Original savin	100	400	100
Podophyllotoxin	0.20	2	40
Savinin	0.10	>1000	0
Xylene mother liquor	2.3	25	37

[a] Minimum effective dose for a single subcutaneous injection in mice bearing implants of Sarcoma 37. [b] Yield × 400/MED. The failure of the last three items in this column to add up to 100% is due to accumulated losses in the fractionation and to large uncertainties in the values of MED.

(7) A series of crystalline waxes called "etholides," probably linear polyesters of ω-hydroxy fatty acids, have been isolated from *J. sabina* and other conifers [J. Bougault and L. Bourdier, *Compt. rend.*, **147**, 1311 (1908); *J. pharm. chim.*, **29**, 561 (1909)]. One of these, m.p. 67–69°, obtained by us from savin, had no activity against Sarcoma 37 in mice.

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Biosynthesis of Carotene in Phycomyces

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The effect of β-ionone on β-carotene production in *Phycomyces*[1] raises many questions as to the course of carotenoid biosynthesis. While it seems most probable that the β-ionone may be incorpo-

(1) G. Mackinney, T. Nakayama, C. D. Buss and C. O. Chichester, THIS JOURNAL, **74**, 3456 (1952).