Compound G.—The chloroform extract of the aqueous phase from the original extraction gave, on evaporation, 57 g. of brown resin. This was extracted with warm benzene to give 3 g. of benzene-soluble gum which was chromatographed on 40 g. of alumina. The fractions eluted with benzene gave a total of 18 mg. of cream colored prisms on crystallization from methanol–ether. Recrystallization from acetone–petroleum ether gave slender colorless prisms, m.p. 216–219°; $[\alpha]_D$ +20 ± 2° (An); ultraviolet spectrum: λ_{max}^{EtOH} 240 mμ (ε × 10^{-3} 14.8), 279 (10.8), 339 (7.4); $\lambda_{max}^{EtOH + NaOH}$ 259 mμ (9.1), 356 (25.1); infrared spectrum (μ): 3.00, 6.04, 6.17, 6.32, 6.62, 6.79, 6.94, 7.35, 7.71, 8.25, 8.64, 8.93, 9.72, 10.74, 11.49, 12.76, 13.29, 14.30.

Anal. Calcd. for $C_{16}H_{14}O_5$ (286.27): C, 67.13; H, 4.93. Found: C, 66.81; H, 5.11; mol. wt. (Rast), 254.

A small sample of the compound was treated with excess diazomethane. The product was crystallized four times from ether–petroleum ether to give white prisms, m.p. 173–178°. The ultraviolet spectrum in ethanol showed the same maxima as the parent compound with somewhat lower extinctions. In alkali the spectrum was quite different; $\lambda_{max}^{EtOH + NaOH}$ (mμ) 229 (E_1^1 595), 249 (462), 272 (358), 338 (297), 397 (410); λ_{min} 304.

Methylene Chloride Extraction. Isolation of Rotenone.—Seven pounds of shredded root bark was extracted with 9 l. of methylene chloride in six portions. The combined extracts were evaporated, and the residue (18 g.) was dissolved in benzene and chromatographed on 180 g. of alumina. The first fraction eluted with benzene furnished 310 mg. of jamaicin, m.p. 192–194°. The intermediate fractions eluted with chloroform gave no crystals. The final fraction, eluted with methanol, deposited a very small quantity of crystals from chloroform, m.p. 270–280°. This was identified as lisetin by the Meijer test and ultraviolet spectrum.

The mother liquors of the jamaicin from the first fraction were then rechromatographed on 87 g. of alumina. The first fraction, 4.5 g. eluted with petroleum ether–benzene (1:1), furnished a further 66 mg. of jamaicin from methanol. The mother liquors from this crop were stored in the ice-box in methanol–ether solution, and slowly deposited a larger second crop of prism-clusters. This material was twice recrystallized from chloroform–petroleum ether, weight 170 mg., m.p. 163–164°. A mixed m.p. with jamaicin gave a large depression, a mixed m.p. with authentic rotenone gave no depression; the infrared spectrum was superimposable upon that of authentic rotenone. Further crystallization of this fraction then gave a small third crop of crystals which were recrystallized from chloroform–petroleum ether to give 25 mg. of jamaicin, m.p. and mixed m.p. 160° (190–191°).

The fractions of this chromatogram eluted with benzene gave 4 mg. of compound D from chloroform–ether, m.p. 236–255° dec., bright violet Meijer test.

Isolation of Piscidic Acid.—One-half of the aqueous solution remaining after the chloroform extraction, containing about 200 g. of solids, was treated with 400 ml. of saturated lead acetate solution. The resulting dark brown precipitate was filtered, dissolved in warm acetic acid, and reprecipitated with water after Norite treatment. The lead salt was then filtered and decomposed with hydrogen sulfide in alcohol suspension. The alcohol was completely evaporated and the dark brown residue was extracted with warm ethyl acetate. This extract was concentrated and diluted with chloroform to precipitate brown, amorphous impurities. After this process had been repeated several times, the ethyl acetate was evaporated and the oily residue partially crystallized. The solid was separated from the oil and sublimed at 140° (0.05 mm.). Less than 1 mg. of sublimate was obtained, m.p. 181–185°, $\lambda_{max}^{H_2O}$ 223 mμ (A:5.55), 274 mμ (A:0.985); $\lambda_{max}^{0.01\ N\ NaOH}$ 240 mμ (A:6.95), 292 mμ (A:14.8).

DETROIT 32, MICHIGAN

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Senecio Alkaloids. The Composition of "Hieracifoline" and "Jacobine"

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The alkaloid "hieracifoline," isolated by Manske from *Erechtites hieracifolia*, is a mixture. By employing a partition chromatographic procedure, the two compounds, namely, senecionine and seneciphylline, were separated. From the alkaloid "jacobine," isolated by Manske[1] from *Senecio jacobea*, senecionine, seneciphylline and a third alkaloid which is identical with Bradbury's and Culvenor's[6] jacobine, were obtained. These same three components in different ratios were present in a sample of *Senecio jacobea* L. of Norwegian origin.

(A) The Alkaloids from *Erechtites hieracifolia*.—Manske[1] reported in 1939 the isolation from *Erechtites hieracifolia* of an alkaloid $C_{18}H_{25}NO_5$ isomeric with senecionine, integerrimine and usaramoensine. This alkaloid, m.p. 227°, $[\alpha]_D$ −89.7°, was named hieracifoline. It gave on hydrolysis the base retronecine and an acid $C_{10}H_{16}O_5$, called hieracinecic acid, isomeric with senecic, integerrinecic, usaramoensenecic and platynecic acids.

From *Senecio aquaticus*, an alkaloid, $C_{18}H_{25}NO_5$, m.p. 220°, $[\alpha]^{15}D$ −83° was reported.[2] This substance, which was named aquaticine, showed characteristics very close to those of the so-called hieracifoline.

Recently Culvenor[3] has shown that the alkaloid pterophine, m.p. 227°, $[\alpha]_D$ −88.5°, obtained first by De Waal[4] from *Senecio pterophus* and *Senecio ilicifolius* is actually a mixture of the two known alkaloids senecionine and seneciphylline. In the same Communication[3] he postulated that hieracifoline is probably a mixture of senecionine and seneciphylline on the basis of the infrared spectra and the R_f values.

The infrared spectra and R_f values, however, do not always permit distinction between stereoisomers. Hieracifoline which was extracted from *Erechtites hieracifolia* was therefore subjected in this Laboratory to further investigation. The melting point and infrared spectrum of the sample available were identical with those of a sample of hieracifoline kindly supplied by Dr. Manske.

Senecionine has a band in the infrared spectrum at 757 cm.$^{-1}$ which was used for identification of this alkaloid in mixtures containing seneciphylline, retrorsine and riddelline.[5] This band, however, is also present in the spectra of the stereoisomers integerrimine and usaramoensine. Moreover, it has now been found that integerrimine, usaramoensine and senecionine all have the same R_f values. For

(1) R. H. F. Manske, *Can. J. Research*, **17B**, 8 (1939).
(2) W. C. Evans and E. T. Evans, *Nature*, **164**, 30 (1949).
(3) C. C. J. Culvenor, *Chemistry and Industry*, 1386 (1951).
(4) H. L. De Waal, *Nature*, **146**, 777 (1940).
(5) R. Adams and T. R. Govindachari, THIS JOURNAL, **71**, 1956 (1949).

TABLE I

Jacobine ($C_{18}H_{25}NO_6$)[a]	M.p., °C.	$[\alpha]_D$	R_f	Found C	Found H	Found N
Senecio jacobea[7]	223–224	....	0.44, 0.58, 0.62	63.83	7.24	4.35
Senecio jacobea[b,7]	225	−38.0	.44	61.38	7.42	..
Senecio jacobea L.[8]	219	−46.3	..	62.1	7.1	4.1
Senecio cineraria[8]	219	−30.5	..	61.8	7.2	4.3
Senecio jacobea L.[6]	226	−40.0	.39	61.8	6.9	4.4

[a] The theoretical values of C, H and N, for the three alkaloids found in Manske's "jacobine" are: $C_{18}H_{23}NO_5$ (seneciphylline): C, 64.85; H, 6.95; N, 4.20. $C_{18}H_{25}NO_5$ (senecionine): C, 64.46; H, 7.51; N, 4.18. $C_{18}H_{25}NO_6$ (jacobine): C, 61.52; H, 7.17; N, 3.98. [b] Values reported are those obtained in this research after partition chromatography of Manske's total alkaloid to eliminate senecionine and seneciphylline and subsequent crystallization from ethanol. The *Senecio jacobea* L. from Norwegian sources gave the same rotation, m.p. and R_f. *Anal.* C, 61.38; H, 7.17; N, 4.14.

identification of the alkaloids in the mixture, hieracifoline was subjected to partition chromatography. Senecionine and seneciphylline were obtained, thus confirming Culvenor's postulation.

A mixture of seneciphylline and senecionine was prepared in the ratio (60% senecionine, 40% seneciphylline) suggested by the values of the optical rotations of hieracifoline on one hand and of senecionine and seneciphylline on the other. This mixture showed a melting point and infrared spectrum identical with those of hieracifoline.

(B) The Alkaloids from *Senecio jacobea* L.—Bradbury and Culvenor[6] have reported the isolation of five alkaloids from *Senecio jacobea* L. One of these alkaloids, which they called jacobine, was believed to be identical with the "jacobine" described by Manske[7] and with that described by Barger and Blackie.[8]

Dr. Manske kindly furnished a sample of "jacobine" which he had isolated from *Senecio jacobea*. It proved to be not a pure compound but a mixture of three alkaloids. A paper chromatogram and the infrared spectrum suggested that two of the components might be senecionine and seneciphylline (or stereoisomers thereof) and the third one—on the basis of R_f values—probably the jacobine described by the Australian workers.

By partition chromatography, three components of Manske's "jacobine" were separated; two of them were senecionine and seneciphylline. The main component was seneciphylline; second in amount was a compound which analyzed correctly for $C_{18}H_{25}NO_6$. Its melting point and R_f value compared favorably with those of Bradbury's and Culvenor's jacobine; senecionine was present as the minor alkaloid.

Another sample of crude extract from *Senecio jacobea* L. of Norwegian origin[9] was also submitted to partition chromatography. The main component of the mixture, accounting for about 60% of the total weight, was a compound which analyzed correctly for $C_{18}H_{25}NO_6$. Its rotation, melting point, R_f value and infrared spectrum were identical with those of the compound $C_{18}H_{25}NO_6$ obtained from Manske's alkaloidal mixture from *Senecio jacobea*. Seneciphylline and senecionine were present in the ratio of about 2.3:1. Hydrolysis of the alkaloid $C_{18}H_{25}NO_6$ of Norwegian origin yielded retronecine and jaconecic acid. From the results of the hydrolysis and the similarity of the physical properties, it may be deduced that the alkaloid is identical with the jacobine described by Bradbury and Culvenor and with that obtained from Manske's alkaloidal mixture.

The constants for the alkaloidal material called jacobine, isolated by Barger and Blackie from *Senecio jacobea* L. and from *Senecio cineraria*, are compared in Table I with those of jacobine described by Manske and by Bradbury and Culvenor.

The R_f values determined during this investigation were obtained at 24 ± 1° using the descending technique and butanol–acetic acid as the solvent. Bradbury and Culvenor used the ascending technique at 17 ± 1°, and the same solvent mixture. The values obtained by the technique used in this Laboratory are consistently a little higher than those reported by the Australian workers.

The alkaloidal material which Barger and Blackie obtained from *Senecio jacobea L.* and from *Senecio cineraria* was probably in both cases a mixture.

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Experimental

Extraction of Hieracifoline.—The alkaloidal material was obtained by extraction of *Erechtites hieracifolia* according to Manske.[1] The crude material was crystallized from chloroform–methanol; the product melted at 226–227°; 0.035 g. made up to 1.5 ml. with chloroform at 25° gave α_D −2.07, l 1; $[\alpha]^{25}D$ −88.8°; its infrared spectrum was identical with that of a sample of "hieracifoline" kindly supplied by Dr. Manske.

Paper Chromatography.—The papergrams were run with the descending technique on Whatman No. 1 paper (22 × 18 inches), using butanol–acetic acid as a solvent. This was the upper part resulting from shaking butanol with an equal volume of aqueous acetic acid (5%). The equilibration and development periods were 24 and 28 hours, respectively. The paper was installed in an all-glass apparatus, situated in a room at constant temperature (24 ± 1°). The spots were developed by means of iodine vapors. Papergrams were run simultaneously of integerrimine, senecionine, seneciphylline, hieracifoline, Manske's alkaloidal mixture from *Senecio jacobea*, a crude extract of *Senecio jacobea* L. of Norwegian origin, and a 1:1 mixture of seneciphylline and senecionine. The mixtures of seneciphylline and senecionine, both natural and artificial, gave spots which were always much more elongated than those due to either senecionine or seneciphylline; however, resolution of these spots was not obtained in every run. The R_f values obtained in a typical successful run are indicated in Table II.

Partition Chromatography of "Hieracifoline."—A portion of 54 g. of Celite 545 was ground in a mortar with 50% of its weight of a molar solution of $NaH_2PO_4 \cdot H_2O$ and then enough solvent (80% CCl_4–20% $CHCl_3$ saturated with molar

(6) R. B. Bradbury and C. C. J. Culvenor, *Chemistry and Industry*, 1021 (1954).

(7) R. H. F. Manske, *Can. J. Research*, **15**, 651 (1931).

(8) G. Barger and J. J. Blackie, *J. Chem. Soc.*, 584 (1937).

(9) This material was extracted by Prof. N. A. Sörensen in the Laboratories of the Organic Institute, Technische Hochschule, Trondheim, Norway. The authors are very grateful for this gift.

TABLE II

Alkaloid	*Rf* (Butanol–acetic acid)[a]
Integerrimine	0.61
Senecionine	.62
Seneciphylline	.58
"Hieracifoline"	.58, 0.62
Manske's alkaloidal mixture from *Senecio jacobea*	.44, *.58, 0.62*
Crude extract of *Senecio jacobea* L. from Norway	*.44*, .58, .62

[a] Numbers in italics indicate spots present in major amount.

phosphate solution) was added to give a freely moving slurry. The material was packed into a glass column (2.2 × 54 cm.) using a close fitting perforated plunger. A solution of 40 mg. of "hieracifoline" in about 5 ml. of chloroform was poured on about 1 g. of Pyrex glass powder and the solvent was eliminated in a vacuum. The glass powder was then packed on top of the chromatographic column. Elution was begun with a mixture of carbon tetrachloride and chloroform (4:1). The first twenty-three 5-ml. fractions contained no dissolved material. A solvent mixture of 3:2 of carbon tetrachloride and chloroform was then used. Six 5-ml. fractions contained nothing, but the succeeding fractions 30 to 38, gave a white powder, which after one crystallization from benzene proved to be pure senecionine.

The next five fractions contained no material. The elution was then continued with pure chloroform and seneciphylline was obtained, which after one crystallization from benzene was pure. A mixture of 13 mg. of senecionine and 9 mg. of seneciphylline (ratio 3:2) was dissolved in chloroform and the solvent was eliminated at room temperature. The melting point of the mixture was 227° with slight decomposition at lower temperature. The infrared spectrum of such a mixture was identical with that of "hieracifoline."

Partition Chromatography of Manske's Alkaloidal Mixture from Senecio jacobea.—A chromatographic column was prepared as above using 80 g. of Celite 545. On top of the column 0.126 g. of Manske's total alkaloid was placed by the usual procedure. Elution was performed with mixtures of: (a) 60% carbon tetrachloride, 40% chloroform (fractions 0–41); (b) 20% carbon tetrachloride, 80% chloroform (fractions 42–79); (c) chloroform (fractions 80–100). 3-Ml. fractions were collected. The results are indicated in Table III.

TABLE III

Fractions	*Rf* value	Res. on evap., mg.	M.p., °C.
0–27	..	None	
28–36	0.62	20	244
37–41	..	None	
42–56	.58	55	217
57–66	..	None	
67–73	.44	35	225
74–100	..	None	

The infrared spectra of material from fractions 28, 31 and 36 and from 42, 49 and 56 showed that these consisted of practically pure senecionine and seneciphylline, respectively. The combined fractions 67–73 yielded jacobine which was crystallized once from 95% ethanol, m.p. 225°; 0.025 g. made up to 1.5 ml. with chloroform at 25° gave α_D −0.63°, *l* 1; $[\alpha]^{25}D$ −37.8°. For analysis, see Table I.

Partition Chromatography of Crude Extract from *Senecio jacobea* L. of Norwegian Origin.—A chromatographic column was prepared as above using 80 g. of Celite 545. On top of the column was placed 1.2 g. of crude extract by the usual procedure. Elution was performed with a mixture of 60% CCl_4 and 40% $CHCl_3$; 5-ml. fractions were collected. The results obtained are indicated in Table IV.

TABLE IV

Fractions	*Rf* value	Res. on evap., g.
26–32	0.58, 0.62	0.365
33	.44, .58, 0.62	.052
34–35	.44	.114
36–86	.44	.545

Fractions 26–32 were combined and chromatographed again according to the procedure described above for the separation of "hieracifoline" into its components. The results showed that fractions 26–32 contained about 30% of senecionine and 70% of seneciphylline. Fractions 34–86 were reunited after the determination of infrared spectra on several fractions indicated the presence of only a single compound. Crystallization from 95% ethanol yielded colorless plates, m.p. 225–226°; 0.0274 g. made up to 2.0 ml. with chloroform at 25° gave α_D −0.52°, *l* 1; $[\alpha]^{25}D$ −38°. For analysis, see Table I, footnote *b*.

Aqueous Alkaline Hydrolysis of Jacobine.—The alkaloid was hydrolyzed with aqueous barium hydroxide by the usual procedure[10] and the acid and basic fragments isolated: (a) The ether extract of the hydrolysis product after acidification yielded, on recrystallization from ether, white needles, m.p. 182–183°; 0.020 g. made up to 2.0 ml. with 95% ethanol gave α_D +0.30°, *l* 1; $[\alpha]^{25}D$ +30°.

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.72; H, 6.94. Found: C, 51.72; H, 7.22.

The acid gave no depression of melting point on admixture with an authentic sample of jaconecic acid, kindly supplied by Dr. Manske. (b) From the aqueous solution left after ether extraction, retronecine hydrochloride was isolated in the usual way, m.p. 161–162°.

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(10) R. Adams, K. E. Hamlin, C. F. Jelinek and R. F. Phillips, THIS JOURNAL, **64**, 2760 (1942).