Table I.—Comparison of the Effect of Tablet Excipients

Sample[a]	Standard, Mg.	Control Reading	Sample[b] Reading	Theoretical, Mg.	Found, Mg.	Recovery, %
1	0.5	20.0	10.0	1.00	1.00	100
2	0.5	20.0	13.26	0.75	0.754	100.5
3	0.5	20.0	20.18	0.50	0.497	99.5
4	0.5	20.0	29.92	0.25	0.251	100.5

[a] Each sample contained 65 mg. of excipient mixture.
[b] The sample readings shown are the average of 5 readings.

Assay Method for Solutions of Diethylstilbestrol in Oil.—Solutions of this nature are usually prepared by dissolving the diethylstilbestrol in a vegetable oil such as sesame oil.

For experimental purposes such a solution was prepared by dissolving 50 mg. of diethylstilbestrol in 50 cc. of sesame oil. Different methods of extraction were tried and the following one was found to give quantitative results.

Place sufficient of the oil to give approximately 5 mg. of diethylstilbestrol in a small separatory funnel and add 30 cc. of light petroleum ether. Extract three times with *N*/1 sodium hydroxide, using 15 cc., 10 cc., 10 cc. Combine the alkaline extracts, acidify with 10% sulfuric acid and extract three times with ether, using 20 cc., 15 cc., 10 cc. Combine the ether extracts and evaporate to dryness. Dissolve the residue in 10 cc. of methanol and apply the color reaction to an aliquot representing 0.5 mg. of diethylstilbestrol as described under Color Test.

Results obtained with this method showed an average deviation of ±1%.

Assay Method for Suppositories of Diethylstilbestrol.—The suppositories used in these experiments were of the glycerine-gelatine type. Several methods of extraction were tried and finally the following one was used.

Place a number of suppositories equal to approximately 5 mg. of diethylstilbestrol in a separatory funnel, add 50 cc. of hot water and agitate until dissolved. Cool and extract three times with ether using 20 cc., 15 cc., 10 cc. Combine ether extracts and extract twice with 15 cc. of *N*/1 sodium hydroxide. Acidify the combined alkaline extracts with 10% sulfuric acid and extract three times with ether using 15 cc., 10 cc., 10 cc. Combine the ether extracts and evaporate to dryness. Dissolve the residue in 10 cc. of methanol and take an aliquot equal to 0.5 mg. of diethylstilbestrol and treat as described under the Color Test.

Results obtained with this method showed deviations of 5%.

SUMMARY

A color reaction for the quantitative estimation of diethylstilbestrol in tablets, ampuls and suppositories has been described.

This reaction is not specific; it is applicable to preparations in which stilbestrol is known to be the only phenyl derivative present.

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A Critical Study of the Physico-Chemical Properties of Tyrolean Oil *Pinus Pumilio* Haenke (Oil *Pinus Montana* Miller) Fam. Pinaceae and Oil *Abies Alba* Miller (Oil *Abies Pectinata* D. C.) Fam. Pinaceae*

By Charles H. Grimm† Edward E. Langenau† and Ernest S. Guenther‡

During recent years, the properties of shipments of guaranteed purity of Oil *Pinus Pumilio* Haenke (Oil *Pinus Montana* Miller) (Fam. *Pinaceæ*) and Oil *Abies Alba* Miller (Oil *Abies Pectinata* D. C.) (Fam. *Pinaceæ*) received from the Tyrol were found to differ considerably from the limits set forth by the standard accepted literature (1) and in the case of Oil *Pinus Pumilio* (Oil of Dwarf Pine Needles) from the standards of the United States Pharmacopœia. This research was undertaken to study the physical and chemical properties of authentic samples of Oil *Pinus Pumilio* and Oil *Abies Alba* from the Tyrol which were distilled under the instruction of our Research Department in different locations, at different times and under different conditions of weather, plant material, etc.

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E. S. Guenther, in 1936, in his survey of Tyrolean oils (2) first pointed out that a change from the reported literature had occurred in the boiling ranges of Oil *Pinus Pumilio* and Oil *Abies Alba*. Schimmel & Co. in their *Annual Reports* (3) mentioned that they had noticed no such change as regards Oil *Abies Alba* and commented, "if 40% of such an oil passes over below 170° C. it has probably been adulterated with Turpentine Oil." The results of the following investigation show definitely that a change in the boiling range of genuine oils has actually occurred.

EXPERIMENTAL

A. Methods.—The samples of Oil *Pinus Pumilio* and Oil *Abies Alba* were examined using the following methods and procedures:

Specific Gravity was determined in a 10-cc. conical pycnometer with a ground-in thermometer. In the case of Oil *Pinus Pumilio*, the gravities are given at 25° C./25° C. since this oil is official in the U. S. P. XI. For Oil *Abies Alba*, the gravities are given at 15° C./15° C. in accord with most scientific work on essential oils.

Optical Rotation was determined in a 100-mm. tube with a Schmidt and Hansch half-shadow polarimeter using a monochromatic sodium vapor lamp as a light source. The rotations for Oil *Pinus Pumilio* were determined at 25° C.; for Oil *Abies Alba*, at 20° C.

Solubility was determined in a 10-cc. glass-stoppered cylinder. The temperature was adjusted to 25° C. for Oil *Pinus Pumilio* and to 20° C. for Oil *Abies Alba* by immersion in a water bath of suitable temperature.

Refractive Index was determined with an Abbé type refractometer. All readings were made at 20° C.

Acid Value was determined with a 5-Gm. sample dissolved in Alcohol and titrated with 0.1*N* NaOH.

Ester Content was determined using a 5-Gm. sample and 0.5*N* NaOH. Refluxing on a steam bath was continued exactly 1 hour; the flask was then permitted to cool for 15 minutes before titrating with 0.5*N* HCl. Esters were calculated as Bornyl Acetate.

Total Alcohol Content was determined by acetylation (1 hour) with subsequent saponification. Total alcohols were calculated as Borneol using the following formula:

$$\text{Total Borneol} = \frac{A \times 7.71}{B - (A \times 0.021)} \times (1 - (E \times 0.0021))$$

where A = cc. of 0.5*N* NaOH consumed
B = weight of acetylized oil (in grams)
E = percentage of ester, calculated as Bornyl Acetate

Table I.—Conditions of Distillation of Oil *Pinus Pumilio*, Haenke (Fam. Pinaceæ)

Sample	General Climatic Condition	Soil	Approx. Age of Tree	Yield of Oil, %	Distillation Material
1	Sunny	Stony	Old	0.24	Needles only—dry
2	Sunny	Stony	Old	0.29	Branch tops with needles (cut to 1 cm.)—dry
3	Sunny	Stony	Old	0.29	Branch tops with needles (cut to 1 cm.)—dry
4	Sunny	Stony	Young	0.36	Wood (branches of 3 cm. diameter cut to 1 cm. *without* needles)—dry
5	Sunny	Stony	Young	0.23	Needles only—dry
6	Sunny	Stony	Old	0.37	Wood (branches of 3 cm. diameter cut to 1 cm. *without* needles)—wet
7	Sunny	Stony	Old	0.34	Small branches with needles
8	Sunny	Stony	Old	0.36	Small branches with needles
9	Sunny	Stony	Old	0.30	Needles only
10	Sunny	Gravel	Old	0.29	Small branches with needles
11	Sunny	Gravel	Young	0.29	Small branches with needles
12	Sunny	Gravel	Young	0.30	Small branches with needles
13	Sunny	Gravel	Old	0.31	Small branches with needles
14	Sunny	Gravel	Old	0.29	Small branches with needles
15	Sunny	Gravel	Old	0.29	Small branches with needles
16	Sunny	Stony	Young	0.28	Small branches with needles
17	Sunny	Stony	Young	0.30	Small branches with needles
18	Sunny	Stony	Old	0.23	Small branches with needles
19	Shady	Stony	Old	0.25	Needles only—dry
20	Shady	Stony	Old	0.27	Branch tops with needles—dry
21	Shady	Stony	Old	0.25	Branch tops with needles—dry
22	Shady	Gravel	Young	0.26	Branch tops with needles—dry
23	Shady	Gravel	Young	0.28	Branch tops with needles—dry
24	Shady	Gravel	Young	0.27	Branch tops with needles—dry

Note: All samples were distilled from trees growing in the Tyrol region at an altitude of approximately 1800 meters. The distillations were continued for 5 hours, the average pressure in the still being 0.3 atmosphere above normal.

Table II.--Properties of Oil *Pinus Pumilio*, Haenke (Fam. Pinaceæ)

Sample	D. 25° C.	α_D 25° C.	n_D 20° C.	Solubility 25° C.	Acid Value	Ester (as Bornyl Acetate), %	Total Alcohol (as Borneol), %	Aldehyde (as Caproic), %
1	0.856	−12° 25′	1.4778	Hazy up to 10 vols. 90% alc.	0.7	4.1	5.0	0.27
2	0.857	− 9° 13′	1.4768	Hazy up to 10 vols. 90% alc.	0.8	4.1	5.2	0.29
3	0.864	− 6° 23′	1.4780	8 vols. 90% alc. A.M.	0.7	5.2	8.0	0.12
4	0.856	−13° 33′	1.4776	Hazy up to 10 vols. 90% alc.	0.8	4.4	4.8	0.01
5	0.864	− 5° 38′	1.4780	6.5 vols. 90% alc. A.M.	0.6	5.7	6.4	0.01
6	0.864	− 6° 52′	1.4779	5.5 vols. 90% alc. A.M.	0.9	5.5	6.1	0.16
7	0.861	−11° 15′	1.4775	Hazy up to 10 vols. 90% alc.	1.1	7.2	8.7	0.31
8	0.862	− 3° 47′	1.4787	Hazy up to 10 vols. 90% alc.	0.4	4.8	5.5	0.14
9	0.858	−14° 6′	1.4769	Hazy up to 10 vols. 90% alc.	0.7	5.9	8.0	0.12
10	0.871	−10° 45′	1.4766	Hazy up to 10 vols. 90% alc.	0.9	9.5	9.3	0.40
11	0.860	−12° 46′	1.4790	Hazy up to 10 vols. 90% alc.	1.3	4.4	5.0	0.17
12	0.862	− 8° 13′	1.4784	Hazy up to 10 vols. 90% alc.	0.5	4.7	4.2	0.15
13	0.862	−10° 4′	1.4781	Hazy up to 10 vols. 90% alc.	0.5	4.1	5.5	0.06
14	0.860	− 6° 5′	1.4779	Hazy up to 10 vols. 90% alc.	0.4	3.9	5.1	0.19
15	0.861	−13° 1′	1.4750	Hazy up to 10 vols. 90% alc.	0.2	3.3	6.7	0.47
16	0.862	− 3° 19′	1.4786	Hazy up to 10 vols. 90% alc.	0.2	4.1	5.6	0.02
17	0.862	− 7° 4′	1.4760	Hazy up to 10 vols. 90% alc.	0.3	4.2	10.3	0.17
18	0.857	−12° 30′	1.4769	Hazy up to 10 vols. 90% alc.	1.0	6.5	8.6	0.09
19	0.854	−13° 2′	1.4770	Hazy up to 10 vols. 90% alc.	0.9	4.7	9.1	0.24
20	0.856	−13° 46′	1.4770	Hazy up to 10 vols. 90% alc.	0.8	5.8	6.8	0.09
21	0.857	−13° 29′	1.4766	Hazy up to 10 vols. 90% alcs.	0.8	6.0	6.7	0.17
22	0.858	−12° 17′	1.4768	Hazy up to 10 vols. 90% alc.	0.7	6.2	7.9	0.16
23	0.857	−15° 16′	1.4766	Hazy up to 10 vols. 90% alc.	0.9	5.9	7.3	0.16
24	0.855	−15° 5′	1.4769	Hazy up to 10 vols. 90% alc.	0.9	5.4	7.2	0.23

Aldehyde Content for Oil *Pinus Pumilio* was determined by means of 0.5 *N* Hydroxyamine Hydrochloride Solution using Brom Phenol Blue indicator. Titration of the liberated HCl after 30 minutes was made with 0.1*N* NaOH. Aldehyde was calculated as caproic aldehyde.

Boiling Range was determined by carefully distilling a 50-cc. sample of the oil in a four-bulb Ladenburg flask (diameter of main bulb = 70 mm., of first refluxing bulb = 45 mm., of second refluxing bulb = 40 mm., of third refluxing bulb = 35 mm.). Anschutz thermometers were used throughout, obviating any stem corrections. Distillations were carried out at atmospheric pressure.

Odor Comparisons were made with standard authentic type samples.

B. Results.—The conditions of distillation, the analyses and the fractionations of twenty-four samples of Oil *Pinus Pumilio* are given in Tables I, II and III.

The conditions of distillation, the analyses and the fractionations of fifteen samples of Oil *Abies Alba* are given in Tables IV, V and VI.

C. Discussion of Results.—Oil of *Pinus Pumilio*. For the sake of comparisons and in order to present a clearer picture of our data, the limits of the standard literature are given in Table VII. It may be seen from our analyses of genuine oils that many of these Tyrolean oils failed to meet the limits as outlined in the literature.

As to the limits of Gildemeister and Hoffmann, "Die Ätherischen Öle," Sample 10 failed to comply with the requirement for specific gravity; Samples 8 and 16, the requirement for optical rotation; Samples 7 and 11, the requirement for acid value; Sample 10, the requirement for ester content; and

Table III.—Boiling Ranges of Oil *Pinus Pumilio*, Haenke (Fam. Pinaceæ)

Sample	Up to +160° C., %	+160°–165° C., %	+165°–170° C., %	+170°–175° C., %	+175°–180° C., %	+180°–185° C., %	+185°–190° C., %	+190°–195° C., %	+195°–200° C., %	+200°–205° C., %	+205° C. and Up, %
1	3.8	5.2	21.2	28.0	18.8	4.4	1.2	0.4	0.8	0.8	15.4
2	4.4	4.0	34.4	28.4	8.0	4.4	0.8	0.6	2.0	1.4	11.6
3	0.4	3.8	18.0	24.4	16.8	9.6	2.4	4.4	1.8	3.2	15.2
4	1.2	2.0	20.2	30.4	12.0	8.4	6.2	1.8	2.2	1.8	13.8
5	2.8	1.2	9.2	32.4	18.4	9.6	4.0	4.4	0.4	2.2	15.4
6	3.6	4.2	12.2	24.0	17.8	7.4	5.4	4.2	1.8	0.6	18.8
7	2.4	7.0	21.0	25.6	9.6	4.4	3.2	5.6	3.2	2.4	15.6
8	3.8	0.8	12.8	34.8	17.2	8.4	4.0	1.6	0.8	0.8	15.0
9	3.6	5.2	18.4	30.0	10.8	4.4	3.2	6.0	1.6	2.4	14.4
10	2.0	6.4	18.6	23.2	17.8	5.2	4.0	3.2	2.4	2.0	15.2
11	3.2	4.4	12.0	37.6	7.2	4.4	6.4	4.8	2.4	0.4	0.4
12	1.8	3.8	17.2	26.4	18.8	7.2	4.0	1.6	3.2	2.0	14.0
13	0.4	2.0	12.2	36.0	22.0	6.8	2.2	1.8	0.8	0.6	15.2
14	3.0	3.4	18.4	32.8	15.2	6.4	4.4	2.8	0.8	0.8	12.0
15	5.0	17.4	30.8	18.4	5.4	4.4	6.0	1.2	2.0	0.4	9.0
16	3.2	4.0	16.0	25.6	24.0	8.4	3.6	2.0	0.6	0.2	12.4
17	4.0	5.8	24.0	26.4	8.0	8.0	4.4	0.8	3.6	0.2	14.8
18	0.4	2.0	13.4	22.6	14.0	6.2	6.4	5.8	6.4	4.0	18.8
19	4.0	3.5	24.8	28.8	12.4	6.4	2.4	4.0	0.7	0.6	12.4
20	1.6	3.2	22.4	25.2	19.2	4.8	3.2	2.4	0.4	4.0	13.6
21	2.6	3.0	18.8	30.4	14.8	8.4	6.0	0.4	0.6	1.8	13.2
22	2.0	4.4	21.2	22.4	12.2	4.2	8.9	2.2	0.6	1.2	20.7
23	1.5	7.0	34.0	14.0	10.0	6.4	2.4	2.4	1.2	0.8	20.3
24	3.2	6.0	26.0	24.8	15.6	6.4	2.8	2.0	0.8	2.6	9.8

Table IV.—Conditions of Distillation of Oil *Abies Alba*, Miller (Fam. Pinaceæ)

Sample	General Climatic Condition	Soil	Approx. Age of Tree	Yield of Oil, %	Distillation Material
1	Sunny	Gravel	Young	0.32	Branch tops with needles (cut to 1/2 cm.)—dry
2	Sunny	Gravel	Young	0.10	Wood (branches of 2 cm. diameter cut to 2 cm. *without* needles)
3	Sunny	Gravel	Young	0.27	Branch tops with needles (cut to 1/2 cm.)—dry
4	Sunny	Gravel	Old	0.30	Needles only—dry
5	Sunny	Gravel	Old	0.27	Branch tops with needles (cut to 1 cm.)—dry
6	Shady	Stony	Old	0.10	Wood (branches of 2 cm. diameter cut to 2 cm. *without* needles)
7	Sunny	Stony	Old	0.27	Branch tops with needles (cut to 1 cm.)—dry
8	Shady	Stony	Old	0.30	Needles only
9	Shady	Stony	Old	0.16	Bark only (cut to 1 1/2 cm.)
10	Shady	Damp	Old	0.30	Branch tops with needles (cut to 1/2 cm.)—dry
11	Shady	Damp	Old	0.15	Wood (branches of 3 cm. cut to 1/2 cm. *without* needles)
12	Shady	Damp	Old	0.38	Branch tops with needles (cut to 1/2 cm.)—dry
13	Shady	Damp	Old	0.30	Needles only—dry
14	Shady	Damp	Old	0.15	Bark only (cut to 1/2 cm.)
15	Shady	Damp	Old	0.12	Wood (branches of 3 cm. cut to 1/2 cm. *without* needles)

Note: All samples were distilled in the Tyrol region within two weeks after date of felling. Trees growing at an altitude of 480–850 meters were used. All distillations were continued for 5 hours, the average pressure in the still being 0.5 atmosphere above normal.

all samples, the requirement for boiling range. However, all these samples met the requirements for refractive index and for solubility. Of these twenty-four oils, none met all the requirements set forth by Gildemeister and Hoffmann.

As to the limits of the U. S. P. X, Sample 10 failed to comply with the requirement for specific gravity; Samples 1, 2, 4, 7, 8, 9, 10, 11, 13, 15, 16, 18, 19, 20, 21, 22, 23 and 24, the requirement for optical rotation; Sample 1, 2, 4, 8, 11, 12, 13, 14, 15, 16, 17 and 19, the requirement for ester content; all samples failed to meet the requirement for solubility and boiling range. However, all these samples met the requirement for refractive index. Of these twenty-four oils, none met all the requirements set forth by U. S. P. X.

As to the limits of the U. S. P. XI, Sample 10 failed to comply with the requirement for specific gravity; Samples 1, 4, 8, 9, 11, 15, 16, 18, 19, 20, 21, 22, 23 and 24, the requirement for optical rotation; Samples 1, 2, 4, 8, 11, 12, 13, 14, 15, 16, 17 and 19, the requirement for ester content; Samples 1, 2, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, the requirement for solubility; Sample 15, the requirement for boiling range. Of these twenty-four oils only Samples 3, 5 and 6 met all the requirements set forth by the U. S. P. XI.

As a matter of interest the analyses of shipments (4) of Tyrolean Oil *Pinus Pumilio* received in the last few years are presented in Table VIII.

The U. S. P. X requires a solubility of Oil *Pinus Pumilio* in ten parts of 80% alcohol. Genuine

Table V.—Properties of Oil *Abies Alba*, Miller (Fam. Pinaceæ)

Sample	D. 15° C.	α_D 20° C.	n_D 20° C.	Solubility 20° C.	Acid Value	Ester (as Bornyl Acetate), %	Total Alcohol (as Borneol), %
1	0.874	−47° 36′	1.4740	5.5 vols. 90% alc. A.M.	0.7	6.4	6.7
2	0.874	−28° 35′	1.4751	5.5 vols. 90% alc. A.M.	0.4	4.1	5.5
3	0.877	−46° 35′	1.4742	5.5 vols. 90% alc. A.M.	0.4	8.1	8.0
4	0.880	−47° 4′	1.4745	5 vols. 90% alc. A.M.	0.9	7.1	8.3
5	0.876	−41° 52′	1.4746	5 vols. 90% alc. A.M.	0.9	5.6	9.1
6	0.877	−29° 1′	1.4760	6 vols. 90% alc. A.M.	1.3	0.8	3.6
7	0.879	−43° 47′	1.4749	5 vols. 90% alc. A.M.	0.7	7.6	7.7
8	0.879	−47° 51′	1.4739	5 vols. 90% alc. A.M.	0.3	8.6	8.6
9	0.875	+ 9° 30′	1.4720	5 vols. 90% alc. A.M.	0.2	1.6	3.0
10	0.867	−54° 56′	1.4733	6 vols. 90% alc. A.M.	0.2	3.0	13.4
11	0.867	−58° 12′	1.4751	6 vols. 90% alc. A.M.	0.6	2.3	3.2
12	0.867	−66° 37′	1.4740	6 vols. 90% alc. A.M.	0.3	3.3	6.2
13	0.870	−54° 56′	1.4741	6 vols. 90% alc. A.M.	0.7	2.8	4.3
14	0.878	+ 9° 29′	1.4740	5 vols. 90% alc. A.M.	1.1	0.2	3.3
15	0.870	−13° 30′	1.4760	Hazy up to 10 vols. 90% alc.	1.2	8.2	8.7

Table VI.—Boiling Ranges of Oil *Abies Alba*, Miller (Fam. Pinaceæ)

Sample	Up to +165° C., %	+165°–170° C., %	+170°–175° C., %	+175°–180° C., %	+180°–185° C., %	+185°–190° C., %	+190°–195° C., %	+195°–200° C., %	+200°–205° C., %	+205° C. and up, %
1	23.0	26.0	6.0	8.8	5.6	7.4	0.4	1.6	5.2	16.0
2	36.4	18.8	9.2	4.8	3.4	4.8	2.8	1.2	1.6	17.0
3	20.4	24.0	8.8	8.4	5.4	2.4	4.0	3.2	6.0	17.4
4	24.4	20.4	15.2	8.0	2.0	5.2	2.0	1.2	2.0	19.6
5	32.8	20.0	6.4	4.4	6.8	2.8	2.4	2.0	4.8	17.6
6	31.2	12.8	7.2	7.8	6.4	6.0	6.8	0.8	0.4	20.6
7	27.2	21.6	8.8	3.2	4.4	3.2	2.8	0.8	3.2	24.8
8	34.8	20.8	8.0	4.8	2.0	3.2	4.4	0.8	1.2	20.0
9	44.0	30.4	3.2	3.0	2.0	0.8	2.8	0.8	0.4	12.6
10	20.4	24.0	16.8	7.2	6.5	3.6	2.0	0.8	2.4	16.3
11	13.6	12.0	30.0	12.4	5.2	1.2	2.0	4.4	0.8	18.4
12	19.2	16.4	20.0	10.4	4.4	3.2	1.2	4.0	0.8	20.4
13	21.6	25.2	11.2	8.8	6.4	4.0	2.4	2.0	4.0	14.4
14	44.4	15.2	8.8	8.0	2.0	0.8	3.2	0.4	0.8	16.4
15	24.4	18.0	16.4	8.0	4.8	4.4	3.2	3.2	0.8	16.8

oils do not meet this requirement. They are much less soluble. The Eleventh Revision of the U. S. P. stipulates the solubility of one volume of Oil *Pinus Pumilio* in 4.5 to 8 volumes of 90% alcohol. Generally this does not conform to pure oils since in many cases a clear solution is not obtained.

According to the U. S. P. X and XI Oil *Pinus Pumilio* has an ester content of not less than 5% calculated as Bornyl Acetate. As a matter of fact, however, genuine oils are often below this limit.

As to optical rotation, the U. S. P. X specifies −4° 30′ to −9° in 100-mm. tube at 25° C. These figures are incorrect according to our investigation because genuine Tyrolean oils have an optical rotation from −3° 19′ to −15° 16′. Therefore it is to be regretted that the Eleventh Revision of the U. S. P. requires a rotation of −5° to −12°.

Regarding boiling range, the U. S. P. X states that less than 1% of the oil distils below 165° C. Similarly, Gildemeister and Hoffmann (5) and later the annual reports of Schimmel and Co. allow for only 1% to distil below 165° C. if the oil is to pass as pure. None of these twenty-four authentic oils met this requirement although many of our shipments received during past years have conformed to this requirement. However, these samples conform with the U. S. P. XI, which specifies that less than 10% of the oil distils below 164° C., in the case of all oils examined, except Sample 15. Climatic conditions and other circumstances seem to

Table VII.—Limits of Gildemeister and Hoffmann, the U. S. P. X and the U. S. P. XI for Oil *Pinus Pumilio*

	Gildemeister and Hoffmann	U. S. P. X	U. S. P. XI
Specific gravity	0.860 to 0.875 at 15° C. (0.854 to 0.869 at 25° C.)	0.853 to 0.869 at 25° C.	0.853 to 0.869 at 25° C.
Optical rotation	−4° to −15° 20′	−4° 30′ to −9°	−5° to −12°
Refractive index at 20° C.	1.475 to 1.480	1.4750 to 1.4800	1.4750 to 1.4800
Acid value	Up to 1.0	.....	.....
Ester content (calculated as Bornyl Acetate)	3 to 8%	Not less than 5%	Not less than 5%
Solubility	4.5 to 8 volumes of 90% alcohol, sometimes with turbidity	10 Volumes of 80% alcohol	4.5 to 8 volumes of 90% alcohol
Boiling range:			
Up to +165° C.	Nothing	Less than 1%	Less than 10%
+165° C. to +170° C.	Up to 15%	.....	.....
+170° C. to +175° C.	14 to 40%	.....	.....
+175° C. to +180° C.	12 to 40%	.....	.....
+180° C. to +185° C.	8 to 12%	.....	.....
Above +185° C.	24 to 36%	.....	.....

have a great influence on the properties of Oil *Pinus Pumilio* especially on the boiling range.

In this connection it might prove of interest to list the known constituents of Oil *Pinus Pumilio*.

Known Constituents of Oil Pinus Pumilio:

l-α-Pinene (6)
β-Pinene (7)
l-Limonene (8)
Dipentene (9)
Δ^3-Carene (10)
l-Phellandrene (11)
An aldehyde, $C_{15}H_{26}O$ (12)
A ketone, $C_{16}H_{24}O$ (13)
Caproic aldehyde (14)
Sylvestrene (15)
Cuminic aldehyde (16)
Anisic aldehyde (17)
Δ^2-Isopropyl-4-cyclohexenone (18)
A secondary, unsaturated, monocyclic terpene alcohol, $C_{10}H_{18}O$ (19)
An unsaturated sesquiterpene alcohol, (Pumiliol), $C_{15}H_{26}O$ (20)
Bornyl acetate (21)
Bornyl propionate (22)
Bornyl capronate (23)
A sesquiterpene (cadinene?) (24)
Unidentified tertiary terpene and sesquiterpene alcohols (25)

As can be seen from the above listed constituents, there are present several low boiling terpenes, α-Pinene (boiling point 154° C.), β-Pinene (boiling point 164° C.).

A possible explanation for the apparent change (from the reported literature) in the boiling range is the presence of relatively larger amounts of α- and β-Pinene which in turn might be due to a great number of circumstances; for example, changes in the condition of soil, climate, harvesting, gathering, time of distillation, distillation material (twigs, needles, bark, etc.) and age of tree.

Table VIII.—Limits of Shipments of Tyrolean Oil *Pinus Pumilio*

Specific gravity at 25° C./25° C.	0.857 to 0.864
Optical rotation	−7° to −13°
Refractive Index at 20° C.	1.4750 to 1.4770
Ester content (calculated as Bornyl Acetate)	3.3 to 5.9%
Solubility at 25° C.	Soluble in 5 volumes and more of 90% alcohol; to turbid in 10 volumes of 90% alcohol
Boiling range	Up to 2.8% distils below +165° C.

Oil of *Abies Alba*. The limits of Gildemeister and Hoffmann, "Die Ätherischen Öle" (26) which are generally accepted as standards, are given for Oil *Abies Alba* in Table IX.

Table IX.—Limits of Gildemeister and Hoffmann for Oil *Abies Alba*

Specific gravity at 15° C.	0.867 to 0.886
Optical rotation	−34° to −64°
Refractive index at 20° C.	1.473 to 1.476
Ester (calculated as Bornyl Acetate)	4.5 to 11%
Solubility	Soluble in 4 to 7 volumes of 90% alcohol, sometimes with slight turbidity
Acid Value	Up to 2.0
Boiling Range:	
Below +170° C.	8%
+170° C. to +185° C.	55%

In the following discussion Samples 9 and 14 are excluded, since these two oils were distilled exclusively from bark.

Samples 2, 6, 12 and 15 failed to comply with the requirement of Gildemeister and Hoffmann for

optical rotation; Sample 15, the requirement for solubility; samples 2, 6, 10, 11, 12 and 13, the requirement for ester content; all samples, the requirement for boiling range. However, all these samples met the requirements for refractive index, specific gravity and acid value. Of these thirteen oils, none met all the requirements set forth by Gildemeister and Hoffmann.

As a matter of interest the analyses of shipments of Tyrolean Oil *Abies Alba* received in the last few years are presented in Table X (27).

Table X.—Limits of Shipments of Tyrolean Oil *Abies Alba*

Specific gravity at 15° C. /15° C.	0.873 to 0.876
Optical rotation	−41° to −47°
Refractive index at 20° C.	1.4729 to 1.4749
Ester content (calculated as Bornyl Acetate)	5.2 to 8.5%
Solubility at 20° C.	Soluble in 5 to 10 volumes and more of 90% alcohol
Boiling range.	Up to 41% distils below +165° C. Up to 22% distils between +165° C. to +170° C.

According to our investigation, the boiling ranges obtained for Oil *Abies Alba* differ considerably from the limits reported in the literature. Whereas in the past years Oil *Abies Alba* might have had a boiling range such as indicated in Gildemeister and Hoffmann, oils distilled in recent years give upon distillation a fraction below +170° C. of about 55% and even more. As in the case of Oil *Pinus Pumilio* the same factors may be responsible for this change, since a listing of the known chemical constituents of Oil *Abies Alba* shows the presence of α-Pinene.

Known Chemical Constituents of Oil Abies Alba:

l-α-Pinene (28)
l-Limonene (29)
l-Bornyl acetate (30)
A sesquiterpene (31)
Lauric aldehyde (32)
Decylic aldehyde (33)
Santene (34)

SUMMARY

This investigation of Oil *Pinus Pumilio* and Oil *Abies Alba* has shown that the limits for physico-chemical properties as set forth in the literature are not altogether in agreement with our findings for genuine Tyrolean oils. Therefore, these limits should be revised. On the basis of genuine oils of commerce, these samples of unquestionable purity and the literature, we recommend the following limits for Tyrolean oils:

Oil of *Pinus Pumilio*

Specific gravity at 25° C. /25° C.	0.853 to 0.871
Optical rotation	−3° to −16°
Refractive index at 20° C.	1.4750 to 1.4800
Ester content (calculated as Bornyl Acetate)	3 to 10%
Solubility at 25° C.	Soluble in 4.5 to 10 volumes of 90% alcohol, often with turbidity
Boiling range	Less than 10% distils below +165° C.

Oil of *Abies Alba*

Specific gravity at 15° C./15° C.	0.867 to 0.880
Optical rotation	−13° to −67°
Refractive index at 20° C.	1.4729 to 1.4760
Ester content (calculated as Bornyl Acetate	Up to 11%
Solubility at 20° C.	Soluble in 4 to 10 volumes of 90% alcohol, sometimes with slight turbidity
Boiling range	Since as much as 55% and more distils below +170° C., any definite recommendation would be meaningless

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Some of the Constituents of the Tuber of "Coquí" (*Cyperus Rotundus L.*)

I. Preliminary Examination of the Tuber and Composition of the Fatty Oil

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"Coquí" is the common name given in Puerto Rico to a sedge, perennial by long tuber-bearing rootstocks. It is known in English-speaking countries under the name of nut grass. This sedge is not restricted to this particular tropical area but, on the contrary, it is widely distributed all over the tropical and sub-tropical belts. Often it becomes a pernicious weed (1). Botanically, the plant has been identified as *Cyperus rotundus* L., family Cyperacea (1). The only other plant of this genus to have been phytochemically studied to any extent is *Cyperus esculentus* L. (2).

The tubers of "coquí" are easily available in the fruit markets of Puerto Rico. Our supply came from San Juan, Puerto Rico. When fresh, these tubers have an aromatic odor and possess a bitter-cool taste. In color they are dark brown on the outside and whitish or yellowish within. They have an ovoid shape, measuring from 0.5 to 1.5 cm. in diameter. Each tuber weighs, when fresh, from 1 to 3 Gm.

A water infusion, prepared by boiling the whole tuber, has been used in Puerto Rico for a long time in the treatment of kidney and urinary disorders. It is the common belief that this infusion stimulates diuresis and dissolves urinary stones.

Although the drug is known from very old (3)—the *Kupeiros* of the Greeks—and has been, in the past, official in several pharmacopœias,[1] the literature does not reveal any chemical or pharmacological investigations, either of the tuber[2] or of any other part of the plant. A gross pharmacognostical examination of this tuber has been made by Goebel-Kunze (5).

The present investigation deals with the air-dried drug and consists of an attempt to elucidate the chemical composition of this tuber.

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[1] The Pharmacopœias, in which *C. rotundus* L. tuber is official, are:

Country	Edition	Date
Danish	1	1772
French	1	1818
Mexican	1	1874
"	2	1884
"	3	1896
"	4	1904

It is also recorded in the Chinese Pentsaos, according to Stuart (4), under the name of Hsiang-futzu.

[2] The volatile oil from the tuber has been examined by several investigators. We shall deal with it in a future publication.