

## SUMMARY

1. Certain non-narcotic analgetics alleviate the pain-induced antidiuresis in the hydrated rat.
2. It has been shown that these effects on diuresis are due to the ADS titer of the serum.
3. Hydration appears to increase the toxicity, especially of aminopyrine.
4. Acetylsalicylic acid 600 mg./Kg. (orally, divided dose) blocks the pain-induced TNPSH lowering of rat liver.
5. Further investigations are being made to determine the specificity of these agents in blocking these responses.

## REFERENCES

- (1) Wolff, H. G., and Wolf, S., "Pain," 3rd printing, Charles C Thomas Company, Springfield, Illinois, 1951.
- (2) Wolf, S., and Wolff, H. G., "Human Gastric Function," 2nd ed., Oxford Press, New York, 1947.
- (3) Mirsky, A. I., Stein, M., and Paulisch, G., *Endocrinology*, **54**, 401(1954).
- (4) Karady, S., Browne, J. S. L., and Selye, H., *Quart. J. Exptl. Physiol.*, **28**, 23(1938).
- (5) Howlett, J., and Browne, J. S. L., *Am. J. Physiol.*, **128**, 225(1940).
- (6) Boyd, E. M., Lee, B. K., and Stevens, M. E. T., *Endocrinology*, **32**, 27(1943).
- (7) Dexter, D., Stoner, H. B., and Green, H. N., *Brit. J. Exptl. Pathol.*, **34**, 625(1953).
- (8) Kovacs, K., and Bachrach, D., *Acta Med. Scand.*, **141**, 137(1951).
- (9) Birnie, J. H., Jenkins, R., Eversole, W. J., and Gaunt, R., *Proc. Soc. Exptl. Biol.*, **70**, 83(1949).
- (10) Rosenthal, S. M., *Public Health Repts. (U. S.)*, **58**, 1429(1943).
- (11) Duncan, D. B., and Bonner, R. G., "Simultaneous Confidence Intervals Derived from Multiple Range and Multiple F Tests," Mimeographed report presented to American Statistical Association, Sept. 1954.
- (12) Snedecor, G. W., "Statistical Methods," 4th ed., Iowa State College Press, Ames, Iowa, 1946, p. 406.
- (13) Ginsburg, M., and Heller, H., *J. Physiol.*, **115**, 43(1951).
- (14) Pendergrass, E. P., *Am. J. Roentgenol.*, **46**, 673(1941).
- (15) Jeffers, W. A., Livezey, M., and Austin, J. H., *Proc. Soc. Exptl. Biol. Med.*, **50**, 184(1942).
- (16) Erspamer, V., and Sala, G., *Brit. J. Pharmacol.*, **9**, 31(1954).
- (17) Burn, J. H., Finney, D. J., and Goodwin, L. G., "Biological Standardization," Oxford University Press, London, 1950.
- (18) Register, U. D., and Bartlett, R. G., Jr., *Science*, **120**, 109(1954).
- (19) Lyles, V., Beck, L. V., Linkenheimer, W. H., *Proc. Soc. Exptl. Biol. Med.*, **81**, 291(1952).
- (20) Bartlett, R. G., and Register, U. D., *ibid.*, **83**, 708(1953).
- (21) Bartlett, R. G., and Register, U. D., *ibid.*, **86**, 397(1954).
- (22) Kolthoff, I. M., and Harris, W. E., *Ind. Eng. Chem., Anal. Ed.*, **18**, 161(1946).
- (23) Benesch, R. E., and Benesch, R., *Arch. Biochem.*, **28**, 43(1950).

## Composition of Gum Turpentine of Pines XXVII.\*

### A Report on Three Mexican Pines: *Pinus pringlei* and *P. michoacana* from Michoacan\* and *P. arizonica* from Chihuahua\*

By P. M. ILOFF, Jr., and N. T. MIROV

The gum turpentine of *Pinus pringlei* contained: 73% *d*- and *dl*- $\alpha$ -pinene; 2% *l*- $\beta$ -pinene; 11% *d*- $\Delta^8$ -carene; 1% terpinolene; 2.5% methyl chavicol; 6% *d*-longifolene; and 4.5% pot residue and loss. The gum turpentine of *P. michoacana* contained: 29% *d*- and *dl*- $\alpha$ -pinene; 60% *l*- $\beta$ -pinene; 1-2% *l*- and *dl*-limonene; 2-3% methyl chavicol; 3% *d*-longifolene; and 4.5% pot residue and loss. The gum turpentine of *P. arizonica* contained: 60% *d*- and *dl*- $\alpha$ -pinene; 29% *l*- $\beta$ -pinene; 5% *d*- $\Delta^8$ -carene; 4.2% tails; and 1.8% pot residue and loss.

#### PINUS PRINGLEI SHAW

*Pinus pringlei*, according to Shaw (2), belongs to the group *Insignes*, characterized by the cones that remain closed after the seeds are ripe. It is related to *P. oocarpa*.

*Pinus pringlei* is a subtropical Mexican species growing in the States of Michoacan, México,

Guerrero, and Oaxaca, commonly at altitudes of 1,750 to 1,850 meters above sea level.

The sample of oleoresin used in the present experiment was obtained from Uruapan, Michoacan, through the courtesy of M. Louis Huguet, Forestry Expert of the Food and Agricultural Organization's Mexican project.

#### EXPERIMENTAL

To distill the turpentine, a batch of 2,989 Gm. of oleoresin of *Pinus pringlei* was heated under reduced pressure so that at the end of distillation, temperature reached 190° and the pressure was re-

\* Received September 15, 1955, from the Institute of Forest Genetics, California Forest and Range Experiment Station, maintained by the Forest Service, U. S. Department of Agriculture, in cooperation with the University of California, Berkeley.

The work reported in this paper was aided through a grant from the Rockefeller Foundation.

duced to 2 mm. of mercury. Yield of turpentine was 21 per cent of the weight of the oleoresin.

The crude turpentine of *Pinus pringlei* had the following characteristics:  $d_4^{20}$ , 0.8642;  $n_D^{20}$ , 1.4721;  $[\alpha]_D^{20}$ , +39.5°. A batch of 596 Gm. of the turpentine was fractionated in a 90-cm. long, 25-mm. inside-diameter Todd column packed with 3/32-inch single turn glass helices. A reflux ratio of 10:1 was maintained. The results of the distillation are shown in Table I.

Judging from their physical properties, fractions 2 and 3 contained mainly *d*- and *dl*- $\alpha$ -pinene. A

small yield of *dl*- $\alpha$ -pinene nitrosochloride was prepared from fraction 2. After two recrystallizations from chloroform by addition of cold methanol, the nitrosochloride melted at 103.5–104.5°, and was not depressed in melting point by admixture of authentic *dl*- $\alpha$ -pinene nitrosochloride.

Fractions 4 to 7 were transition fractions containing mostly *d*- and *dl*- $\alpha$ -pinene with some *l*- $\beta$ -pinene and *d*- $\Delta^3$ -carene.

The minimum in optical rotation and maximum in density in fraction 9 indicated the possible presence of *l*- $\beta$ -pinene. Ten grams of fraction 9 was

TABLE I.—FRACTIONAL DISTILLATION OF TURPENTINE OF PINUS PRINGLEI, P. MICHOCANA, AND P. ARIZONICA FROM MEXICO

Fractions	Pressure, mm.	Boiling Range, °C.	Distillate, %	Density		Index of Refraction	Specific Rotation $[\alpha]_D^{20}$
				$d_4^{20}$	$n_D^{20}$		
<i>Pinus pringlei</i> (596 Gm. used) →							
1	760	126–154	0.5	0.856	1.4663	...	
2	760	154–156.5	64.5	0.854	1.4668	+47.0	
3	760	156.5–158	6.7	0.854	1.4672	+44.4	
4	760	158–160	1.0	0.854	1.4689	+36.1	
5	760	160–161	0.4	0.855	1.4697	+31.4	
6	760	161–163.5	0.5	0.855	1.4705	+27.4	
7	760	163.5–164.5	0.5	0.856	1.4718	+21.4	
8	14	56–57	1.1	0.859	1.4732	+13.9	
9	14	57–58	2.2	0.859	1.4742	+10.5	
10	14	58–60	6.2	0.857	1.4745	+12.5	
11	14	60–62	0.9	0.854	1.4768	+10.5	
12	14	62–65	0.7	0.852	1.4788	+8.2	
13	14	65–68	0.5	0.853	1.4812	+6.1	
14	14	68–72	0.5	0.854	1.4841	+4.5	
15	14	72–78	0.4	0.860	1.4871	+2.6	
16	14	78–94	0.7	0.894	1.4962	+3.8	
17	14	94–98	1.4	0.950	1.5131	+5.5	
18	14	98–108	0.8	0.955	1.5181	+11.1	
19	14	108–117	0.3	0.945	1.5068	+32.0	
20	14	117–121	0.6	0.937	1.5020	+28.6	
21	14	121–122	1.0	0.934	1.5022	+46.2	
22	14	122–122.5	2.4	0.932	1.5037	+49.0	
23	14	122.5	1.8	0.930	1.5048	+48.0	
Pot residue			2.2				
Loss			2.2				
<i>Pinus michocana</i> (691 Gm. used) →							
1	760	155	0.1	...	1.3820	...	
2	760	155–156	0.2	0.856	1.4622	+8.4	
3	760	156–158	30.4	0.857	1.4674	+5.8	
4	760	158–160	2.0	0.859	1.4689	+1.9	
5	760	160–161	0.6	0.860	1.4712	-3.9	
6	760	161–162	1.6	0.860	1.4723	-8.4	
7	760	162–163	8.4	0.862	1.4734	-10.8	
8	760	163–164	3.9	0.865	1.4752	-15.6	
9	760	164–165	15.6	0.865	1.4768	-19.8	
10	760	165	13.4	0.866	1.4776	-22.2	
11	760	165	9.7	0.866	1.4781	-22.7	
12	14	51–52	2.0	0.866	1.4781	-22.7	
13	14	52–53	0.6	0.864	1.4783	-22.2	
14	14	53–55	0.3	0.859	1.4777	-21.0	
15	14	55–61	0.2	0.853	1.4777	-20.4	
16	14	61–63	0.4	0.842	1.4773	-19.0	
17	14	63–64	0.3	0.841	1.4774	-16.9	
18	14	64–66	0.1	0.841	1.4773	-15.5	
19	14	66–67	0.3	0.843	1.4784	-13.3	
20	14	67–94	0.3	0.873	1.4798	-6.9	
21	14	94–98	1.2	0.944	1.5052	-1.5	
22	14	98–107	0.6	0.951	1.5076	+1.3	
23	14	107–114	0.4	0.945	1.5045	+12.9	
24	14	114–116	0.4	0.940	1.5025	+27.2	
25	14	116–120	0.8	0.937	1.5020	+40.0	
26	14	120–121	1.7	0.929	1.5028	+44.8	
Pot residue and loss			4.5				

Fractions	Pressure, mm.	Boiling Range, °C.	Distillate, %	Density	Index of Refraction	Specific Rotation
	<i>Pinus arizonica</i> (882 Gm. used) →			$d_4^{24}$	$n_D^{25}$	$[\alpha]_D^{25}$
1	760	to 154	0.4	0.858	1.4623	...
2	760	154-155	0.2	0.854	1.4622	...
3	760	155-156	29.6	0.854	1.4652	+20.5
4	760	156-157	17.0	0.854	1.4659	+18.3
5	760	157-159	8.0	0.855	1.4677	+15.5
6	760	159-161	5.2	0.857	1.4693	+8.3
7	760	161-162	4.6	0.859	1.4706	+3.7
8	760	162-164	2.7	0.860	1.4735	-6.9
9	760	164-165	4.1	0.862	1.4751	-12.2
10	760	165-166	12.4	0.863	1.4760	-14.9
11	16	56-57	3.1	0.864	1.4769	-18.1
12	16	57-59	1.9	0.862	1.4752	-7.5
13	16	59-62	1.9	0.858	1.4738	+2.7
14	16	62-63	1.4	0.857	1.4730	+8.8
15	16	63-64	1.2	0.854	1.4738	+2.7
16	16	64-66	0.5	0.852	1.4752	-1.9
17	16	66-68	0.4	0.850	1.4770	-4.2
18	16	68-70	0.2	0.849	1.4785	-6.4
19	16	70-74	0.4	0.850	1.4811	-6.6
20	16	74-77	0.3	0.852	1.4828	-4.2
21	16	77-85	0.3	0.887	1.4830	-2.5
22	16	85-87	0.5	0.867	1.4704	-4.4
23	16	87-91	0.3	0.879	1.4691	-4.1
24	16	Above 91	1.6	0.937	1.4730	-0.8
Pot residue and loss			1.8			

oxidized with alkaline permanganate (3). The sodium salts (0.3 Gm.) received were recrystallized fractionally; the second crop consisted of about 0.1 Gm. of crystals similar in form to authentic sodium nopinate. These crystals were converted to nopinic acid which was recrystallized from water. A few mg. of white crystals (m. p. 125.5-126.5°) were received. Admixture of authentic nopinic acid did not depress the melting point.

Its odor and physical properties indicated that fraction 10 consisted mainly of  $d$ - $\Delta^3$ -carene. From 5 Gm. of fraction 10, about 0.3 Gm. of crude nitrosate was formed. The nitrosate was recrystallized from chloroform by addition of cold methanol. The nitrosate then melted at 145-146° and the melting point was not depressed by admixture of an authentic nitrosate of  $d$ - $\Delta^3$ -carene.

Judging from their physical properties, fractions 11 and 12 were mainly  $d$ - $\Delta^3$ -carene.

Fractions 13 and 14 were transition fractions containing both carene and terpinolene.

Fraction 15 had properties close to those of terpinolene:

	b. p.°	$n_D$	$d_4$	$[\alpha]_D$
Fraction 15	72-78 (15 mm.)	1.4871 <sup>23</sup>	0.860 <sup>23</sup>	+2.6
Terpinolene <sup>1</sup>	185 (760 mm.)	1.4864 <sup>25</sup>	0.8560 <sup>25</sup>	0.0

From 2 Gm. of fraction 15, 0.5 Gm. of crude crystalline tetrabromide was prepared. After several recrystallizations from ethyl acetate at 0°, a few mg. with m. p. 115-116° were received. The melting point was not depressed by admixture of authentic terpinolene tetrabromide.

Fraction 16 was a transition fraction containing both terpenes and oxygenated compounds.

Fractions 17 and 18 had high refractive indexes and densities which indicated the possible presence of methyl chavicol. A 10-Gm. sample of fractions 17 and 18 was oxidized with acid permanganate and 500 mg. of crude homoanisic acid, m. p. 79.5-81°, was received. After two recrystallizations from water, it melted at 85°; the melting point was not depressed by admixture of authentic homoanisic acid.

The physical properties of fractions 19 to 23 indicated that they consisted mainly of a sesquiterpene. From 3 ml. of fraction 22, 2.0 Gm. of a crude crystalline hydrochloride was prepared. After two recrystallizations from glacial acetic acid, 0.8 Gm. of hydrochloride with m. p. 58.6-59.5° was received. The melting point was not depressed by admixture of the hydrochloride of  $d$ -longifolene.

### PINUS MICHOCANA MARTINEZ

In Shaw's monograph (2), *Pinus michoacana* Martinez is included with *Pinus montezumae*, a heterogeneous complex, whose further study, Shaw said, "would be a valuable contribution to science." Since Shaw's time a great deal of work has been done in studying the *Montezumae* complex, but still more should be done.

*Pinus michoacana* was given the rank of an independent species by Martinez in 1944. This species includes two varieties and two forms. Its typical form occurs in Michoacan, Jalisco, Oaxaca. In Michoacan it may grow with *P. montezumae*, and possibly these two pines cross although no definite information is available on this subject.

A sample of oleoresin of this pine was sent to us by M. Louis Huguet, from Uruapan, Michoacan.

<sup>1</sup> Sutherland, M. D., *University of Queensland Papers, Dept. of Chem.*, 1, No. 34 (1948).

The turpentine was distilled from the oleoresin under reduced pressure so that at the end of distillation, the pressure was 0.1 mm. of mercury and temperature was 190°. Yield of turpentine was 26% of the weight of the oleoresin. The turpentine possessed the following physical characteristics:  $d_4^{25}$ , 0.8657;  $n_D^{25}$ , 1.4760;  $[\alpha]_D^{25}$ , -8.4°.

A batch of 691 Gm. of the turpentine was distilled in a 90-cm. long, 25-mm. inside-diameter Todd column packed with 3/32-inch single turn glass helices. Results are shown in Table I.

The properties of fraction 3 are close to those of  $\alpha$ -pinene. Both the refractive index and the density indicate the presence of a small amount of  $\beta$ -pinene. Seven milliliters of fraction 3 were used to prepare *dl*- $\alpha$ -pinene nitroschloride which, after three recrystallizations from chloroform by addition of cold methanol, weighed 1.2 Gm. and melted at 103–104°. The melting point was not depressed by admixture of authentic *dl*- $\alpha$ -pinene nitroschloride.

The properties of fractions 4 to 7 indicated that they consisted of varying quantities of  $\alpha$ - and  $\beta$ -pinene.

The physical properties of fractions 8 to 12 were close to those of *l*- $\beta$ -pinene. A 20-Gm. portion of fraction 11 was oxidized to sodium nopinate. The sodium nopinate was converted to 1.7 Gm. of nopinic acid which, after one recrystallization from benzene, melted at 125.5–126.5°. The melting point was not depressed by admixture of authentic nopinic acid.

The densities of fractions 12 to 16 approached density of limonene, but the refractive indexes were high.

Fraction 17 appeared to contain mostly *l*- and *dl*-limonene. From 1.9 Gm. of fraction 17, 323 mg. of a tetrabromide, melting at 124–125°, was prepared. The melting point of the tetrabromide was not depressed by admixture of authentic *dl*-limonene tetrabromide.

Fractions 18, 19, and 20 were transition fractions and had properties indicating they were a mixture of terpenes with methyl chavicol.

Fractions 21 and 22 had a high density and refractive index and an odor indicating the presence of methyl chavicol. A 10-Gm. sample of fractions 21 and 22 was oxidized with acid permanganate (1) and 138 mg. of crude white crystals was isolated. After one recrystallization (Darco) from water, the crystals melted at 84–85° and the melting point was not depressed by admixture of an authentic sample of homoanistic acid.

Fractions 23 and 24 had the properties of mixtures of sesquiterpene and methyl chavicol.

Fractions 25 and 26 had properties indicating the presence of *d*-longifolene. From 3 ml. of fraction 26, 1.0 Gm. of sesquiterpene hydrochloride melting at 58–59° was prepared. The melting point of the hydrochloride was not depressed by admixture of a sample of the hydrochloride of *d*-longifolene.

### PINUS ARIZONICA ENGELMANN

*Pinus arizonica* Engelm. was discovered in Arizona, but its main range is in the Mexican states of Sonora, Chihuahua, and Durango. Its variety, *stormiæ* Martinez, occurs in the desert mountains

of Coahuila and adjacent parts of Nuevo Leon. Shaw (2) considers *P. arizonica* as a variety of *P. ponderosa*. Our findings reported in this paper seem to indicate also that these two pines are closely related.

Oleoresin used in the present investigation was received through the courtesy of Sr. Mario González Múzquiz of the Aserraderos González Ugarte S. A., a leading lumber company of Chihuahua. Thanks are also due to Ing. Alfredo Parra R. for supervising the collection of this oleoresin. The sample was accompanied with branches, pieces of bark, and cones, which are deposited in our herbarium. The oleoresin was collected at El Retiro, near San Juanito, Chihuahua, at an altitude of 2,500 meters above sea level.

A batch of the oleoresin, weighing 3,628 Gm., was heated under reduced pressure so that at the end, when all turpentine was distilled off, the pressure was 2 mm. of mercury and temperature reached 180°. The remaining rosin was hard and brittle. The turpentine obtained by this means amounted to 908 Gm., or 25% of the weight of the oleoresin.

The turpentine of *P. arizonica* had the following characteristics:  $d_4^{25}$ , 0.8585;  $n_D^{25}$ , 1.4690;  $[\alpha]_D^{25}$ , +9.7°.

A batch of 882 Gm. of the turpentine was distilled through a 90-cm. long, 25-mm. inside-diameter, Todd column, packed with 3/32-inch single turn glass helices, and equipped with magnetic control of reflux. A reflux ratio of 10:1 was maintained. Results of the distillation are shown in Table I.

The physical properties of fraction 3 indicated that it was mainly *d*- and *dl*- $\alpha$ -pinene. A nitroschloride was prepared from 7 ml. of fraction 3. After four recrystallizations from chloroform by addition of cold methanol, 0.7 Gm. of crystals, melting at 104–105°, was received. No depression in melting point was observed on admixture of authentic *dl*- $\alpha$ -pinene nitroschloride.

Judging from the physical constants, fractions 4 and 5 were also mainly  $\alpha$ -pinene and fractions 6 to 9 contained various proportions of  $\alpha$ - and  $\beta$ -pinene.

The constants of fractions 10 and 11 indicated that they were mainly *l*- $\beta$ -pinene. From alkaline permanganate oxidation of 10 Gm. of fraction 11, 0.7 Gm. of nopinic acid, m. p. 126–126.5°, was received. The melting point was not depressed by admixture of authentic nopinic acid.

Fractions 12 and 13 were transition fractions containing both *l*- $\beta$ -pinene and *d*- $\Delta^3$ -carene. The physical properties indicated that fraction 14 was mainly *d*- $\Delta^3$ -carene. From 5 Gm. of fraction 14, 1.0 Gm. of crude nitrosate was prepared. On recrystallization from chloroform by addition of cold methanol, 0.6 Gm. of crystals, which melted at 144–145°, was received. The melting point was not depressed by admixture of authentic nitrosate of *d*- $\Delta^3$ -carene.

Fractions 16 to 24 contained mixtures of terpenes and possibly some oxygenated compounds which were not identified.

### REFERENCES

- (1) Bertram, J., and Walbaum, H., *Arch. Pharm.*, **235**, 179(1907).
- (2) Shaw, G. R., "The Genus *Pinus*," Publications of the Arnold Arboretum, No. 5. Cambridge, Mass., 1914.
- (3) Wallach, O., *Ann.*, **356**, 228(1907).