(15) Haas, P., and Hill, T. G., "Chemistry of Plant Products" (Fourth edition), 199.
(16) Morrow, C. A., "Biochemical Laboratory Methods" (1927), 234.
(17) Carré, M. H., and Haynes, D., *Biochem. J.*, 16 (1922), 60.
(18) Morrow, C. A., "Biochemical Laboratory Methods" (1927), 179.
(19) Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 100 (1933), 695.
(20) Parry, E. J., "Allen's Commercial Organic Analysis" (Fifth edition), 4 (1925), 220.
(21) Jannke, P. J., *Pharm. Arch.*, 9 (1938), 75.

A Comparative Study of the Total Volatile Acids of Viburnum Stem and Root Barks*

By Irvine W. Grote and Charles Colburn†

Two species of Viburnum are official in N. F. VI, namely, *V. prunifolium* and *V. opulus*. The latter is a small shrub and since its introduction into U. S. P. VII the "dried bark" has been official. *V. prunifolium* was official in U. S. P. VI and VII as the "dried bark." In U. S. P. VIII, the root bark was specified, but this was changed back to the "dried bark" in U. S. P. IX and again changed to "root bark" in N. F. V and VI. In U. S. P. VIII and IX, *V. lentago* could be used as *V. prunifolium*. Examination of the literature does not reveal any scientific reason for this alternate change from *V. prunifolium* whole bark to root bark. Continued collection of the root bark of *V. prunifolium* will lead to a growing scarcity, while, if merely the stem bark is collected, the tree is quickly replaced by shoots from the original root system. This paper represents the first in a series of comparisons by chemical means of the stem and root barks of *V. prunifolium*.

Both Valerian and Viburnum are peculiar in that they are reported to contain large amounts of valeric acid compounds as well as unusually large amounts of other volatile aliphatic acids released upon hydrolysis. We have found in our own laboratory that the bark of the various Viburnums release several times as much volatile acid following hydrolysis as do such barks as *Cascara Sagrada*, etc. Many workers have assumed that the valeric acid compounds are responsible for the uterine sedative action of the two drugs. Heyl and Barkenbus (1) made perhaps the most careful study of *V. prunifolium* root bark and report after hydrolysis the presence of the volatile acids formic, acetic, valeric and traces of butyric.

No simple direct methods for accurate quantitative determinations of the separate aliphatic acids in presence of several others of the same series could be found in the literature. The steam distillation method of D. C. Dyer (2), however, seems to afford a useful and rapid method of comparison of the amount of one mixture of volatile acids with another and to determine any marked variation in composition of the acids in the mixture. The method consists of steam distillation of the acids from a constant volume of solution and titration of successive 100-cc. fractions of the distillate with alkali. Each of the lower aliphatic acids steam distils at a constant rate proportional to the amount of volatile acid still present in the constant volume. The rate for the acids, through caproic at least, is characteristic for the individual acid and is the reverse of what would be expected, caproic acid distilling at the most rapid rate and formic acid at the slowest rate. Mixtures of the acids distil at rates proportional to the amount of each acid present in the mixture.

In view of the apparent importance of the volatile acids in Viburnum bark, and their possible relation to physiological action, it seemed worth while to apply Dyer's method in a comparison of stem and root barks of *Viburnum prunifolium*. The method was modified in that no attempt was made to keep the volume of liquid constant but rather to run the distillations under identical conditions. In addition, Dyer has shown that over twenty successive 100-cc. fractions were required to remove substantially all the formic acid. This would make the method unnecessarily long for comparative purposes. By the fifth successive 100-cc.

* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.
† From the Department of Chemistry, University of Chattanooga, Chattanooga, Tennessee.

fraction all the acids down to propionic are removed, as well as two-thirds of the acetic and one-half of the formic. Since we are largely interested in the higher volatile acids, and then only in comparing one drug with another under like conditions, the distillation was carried through only five 100-cc. portions in one series and through six 100-cc. portions in another. In this latter series, larger flasks and a slightly different technique were used. Both methods gave results differing in the figures obtained but strictly comparable as to results. Only one series is reported in this paper. The procedure used was as follows:

EXPERIMENTAL

Twenty grams of the bark ground to 5–10 mesh are hydrolyzed on a water bath with 100 cc. 5% sulfuric or phosphoric acid in a 275 sulfur flask fitted with a reflex condenser. The same flask, while still hot, is then fitted with a steam inlet tube reaching to the bottom and an outlet tube passing through a Kjeldahl bulb to a condenser. Steam generated in a 1 l. boiling flask is passed in at such a rate that the distillate is collected at about 5 cc. per minute. By arranging the sulfur flask so as to touch the boiling flask, five 100-cc. portions can be conducted over without the flask overflowing. Five successive 100-cc. portions are collected in short 100-cc. cylinders and titrated in a cumulative manner by adding each 100-cc. portions to a 750-cc. flask, using half-normal sodium hydroxide and phenolphthalein indicator. As reference standards, 1 cc. of the aliphatic acids from formic through caproic (Eastman) were added to twenty grams of acid exhausted Viburnum bark and 100 cc. 5% sulfuric acid and the mixture heated for six hours on the water bath and then steam distilled. The results obtained are given in Table I. Mixtures of the acids show changing rates of distillation varying distinctly from those of the individual acids, since the higher acids are more quickly removed. In order to show easily comparable figures, the titration figures in cubic centimeters were also calculated as to percentage distilled in each fraction by the formula

$$\% = \frac{\text{total cc. of } N/2 \text{ NaOH used through this 100-cc. fraction } x\ 100}{\text{total cc. of } N/2 \text{ NaOH used in titration of the five 100-cc. fractions}}$$

Table I.—Steam Distillation Rates of Approximately 1-Cc. Portions of the Lower Aliphatic Acids Mixed with 20 Grams Exhausted Viburnum Bark and 100 Cc. 5% Sulfuric Acid

Acid	Cc. $N/2$ Alkali Required per 100-Cc. Portion						Cumulative % of Total				
	1	2	3	4	5	Total	1	2	3	4	5
Formic	7.2	5.4	4.0	2.3	2.1	21.0	34	60	79	90	100
Acetic	10.9	6.5	3.1	1.8	1.8	24.1	45	72	85	92	100
Propionic	14.3	5.3	2.6	1.0	0.2	23.4	61	83	95	99	100
Technical butyric	13.0	3.2	1.4	0.8	0.2	18.6	70	87	94	99	100
N Butyric	15.8	4.5	1.7	0.8	0.5	23.3	68	90	94	98	100
Isobutyric No. 2	16.0	2.6	0.6	0.2	0.0	19.4	82	96	99	100	...
N Valeric	17.0	2.5	0.6	0.1	0.0	20.2	84	91	99	100	...
Isovaleric	14.4	1.7	0.3	0.0	0.0	16.4	87	98	100	...	...
N Caproic	13.6	1.3	0.3	0.0	0.0	15.2	90	98	100	...	...
Isocaproic	12.5	1.1	0.3	0.0	0.0	13.9	89	97	100	...	...

Table II.—Commercial Root Bark Samples

(Each figure represents an average of two or more determinations on 20 Gm. representative samples.)

Drug	Cc. $N/2$ Alkali per 100-Cc. Frac. Dis.						Cumulative % of Total				
	1	2	3	4	5	Total	1	2	3	4	5
Prunifolium No. 1	11.5	3.5	2.6	2.1	1.7	21.4	53	70	82	92	100
Prunifolium No. 2	9.3	4.9	3.0	1.4	1.0	19.6	47	72	88	95	100
Prunifolium No. 3	9.8	5.7	3.0	1.6	0.8	20.9	47	74	88	96	100
Purnifolium No. 4	9.5	5.3	2.5	1.6	0.9	19.8	46	72	86	94	100

Table III.—Commercial Tree Bark Samples Corresponding to Table II

Drug	Cc. $N/2$ Alkali per 100-Cc. Fraction Distillate						Cumulative % of Total				
	1	2	3	4	5	Total	1	2	3	4	5
Prunifolium No. 1	8.3	4.9	2.7	1.5	1.1	18.5	45	69	86	94	100
Prunifolium No. 2	8.0	3.2	3.1	0.8	0.6	15.7	51	71	91	96	100
Prunifolium No. 3	9.1	4.1	2.6	1.4	0.8	18.0	51	73	88	96	100
Prunifolium No. 4	8.5	4.5	2.4	1.3	0.8	17.5	48	74	89	96	100
Prunifolium rossed bark	10.3	6.6	3.6	2.3	1.8	24.6	42	69	83	93	100

Irregular variations between the *normal* and *iso* forms of the three higher acids are probably due to the fact that both boiling points and solubility in water vary irregularly between the isomers. Mixtures of a higher and a lower acid are detected by the fact that the rate of distillation drops off in the last fractions since the higher acid is practically removed in the first three fractions. Mixtures of formic, acetic and valeric acids, however, can simulate acetic acid as shown by the Viburnum bark distillations given in Tables II and III. Reduction tests indicating formic acid are readily obtained in the Viburnum distillates as well as the odor of the higher acids. The largest amount of acid present seems to be acetic acid.

The *Viburnum prunifolium* barks given in Table II and Table III were representative samples of large commercial lots obtained from four principal dealers, and were authenticated by well-known pharmacognosists.

CONCLUSION

The results given would seem to indicate the following conclusions:

1. Root bark of *Viburnum prunifolium* contains up to some 20% more total volatile acid following hydrolysis than does stem bark.

2. The proportion and kinds of acid present in the mixture are practically the same in the case of root as compared to stem bark. The greater amount present in the former is due to more acid of all kinds rather than more of the higher acids such as valeric. Variation in the distillation rates of root bark differ as widely between individual samples as they do between root and stem bark.

3. One sample of rossed stem bark had more total acidity than did any sample of root bark. A greater proportion of the lower acids seem to be present in this sample, however, than in the unrossed samples or in the root bark, and such treatment may not be wholly desirable.

4. If the amounts and proportions of the mixture of volatile acid present are any measure of activity, there does not seem to be sufficient difference between stem and root bark of *Viburnum prunifolium* to justify that only the root bark be official.

REFERENCES

(1) Heyl and Barkenbus, *J. Am. Chem. Soc.*, 42 (1920), 1744–1755.

(2) Dyer, D. C., *J. Biol. Chem.*, 28 (1917), 445–473.

The Growth Effects of Thiamin Chloride, Ascorbic Acid and Phytohormones on Belladonna and Ricinus*

By Louis C. Zopf†

Data of considerable value are rapidly accumulating from currently widespread experimentation as to the effects of phytohormones; vitamins and related organic stimulants upon seed germination and general growth of plants (1, 5, 7, 8, 13, 15, 17, 18, 20). Most such experiments have dealt with the effects of a single stimulant upon crop plants. Relatively little information is as yet available as to effects of varying combinations of two or more stimulants employed simultaneously or successively. Neither have medicinal plants been employed to any significant extent in such studies.

Most experiments performed by other investigators on the effects of growth stimulants have merely employed seedlings or very young plants. Very few data are available as to the effects of phytohormones and vitamins upon plants permitted to attain maturity.

An experiment was consequently devised to determine the specific effects of certain hormone and vitamin-like stimulants, singly and in combination upon the entire growth cycle of the official species *Atropa Belladonna* and *Ricinus communis*.

EXPERIMENTAL

MATERIALS AND METHODS

The experimental procedure involved (1) the use of vitamin and hormone-like stimulants applied to seeds in various concentrations in a medium of powdered talc (U. S. P.) of 200 mesh in fineness, and (2) the application of aqueous solutions of the stimulants to growing plants in pure quartz sand cultures.

1. Seed Treatment.—Crystalline indole acetic acid and *a*-naphthalene acetic acid were weighed out and added to proper amounts of talc and then made into a uniform mixture by a three-hour treatment in a ball mill. Vitamins B_1 and C were

* Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.

† Assistant Professor of Pharmacy, College of Pharmacy, State University of Iowa.