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APOCYNUM.

The purposes of this investigation were to seek to ascertain the botanical identity of commercial Apocynum, preparations of which have constantly varied in potency, to determine which commercially available species should be represented in the official definition and to make recommendations to the National Formulary Revision Committee for any changes found necessary in the present monograph as a result of these findings.

History.—Apocynum was known to the American aborigines who used the rhizomes and roots of *Apocynum cannabinum* and *Apocynum androsæmifolium* for dropsy, ague and other conditions, and acquainted the early white settlers with its virtues. The aerial stems of these plants were also employed by them as a source of fibers for making cordage, fishing nets and coarse cloth, whence the vernacular name "Indian Hemp."

The drug was first introduced into professional medicine by M. L. Knapp in 1826 (1). It has since been used by many physicians in the treatment of cardiac diseases attendant with dropsy and is listed in the digitalis group of cardiac tonics. Sollmann (2) states that Apocynum is an effective member of the digitalis group, but without serious advantages.

The drug has been recognized in all editions of the U. S. P. up to 1910. The editions of 1820, 1828 and the New York edition of 1830 recognized as the source of this drug, *Apocynum androsæmifolium* or Dogbane. The *Apocynum cannabinum* or Canadian Hemp was first mentioned in the Philadelphia edition of 1830, which, like the editions which followed up to 1870, recognized both *Apocynum cannabinum* and *Apocynum androsæmifolium*. The United States Pharmacopœias of 1880 and 1890 recognized only *Apocynum cannabinum*. The U. S. P. of 1900 recognized *Apocynum cannabinum* and closely related species of *Apocynum*. It was dropped from the pharmacopœia in 1910 and admitted into the National Formulary which has since recognized only *Apocynum cannabinum* as the official source.

While the Formulary has restricted the source to *A. cannabinum*, the observations of the senior author made on numerous samples of commercial drug over a period of more than twenty years have shown it to be considerably variable, usually consisting of a mixture of *A. cannabinum* and *A. androsæmifolium* or of *A. androsæmifolium* only. While about 30 North American species of *Apocynum* have been described, only two good species and doubtlessly their varieties have as far as we are aware been regularly gathered for the American drug market.

The first real attempt toward standardizing this drug biologically was made by Munch and Krantz in 1934. They made fluidextracts from *Apocynum cannabinum* and *A. androsæmifolium* gathered by Prof. W. L. Stoneback and assayed each preparation by the one hour frog method. They showed that fluidextracts from each of these species had precisely the same physiological activity and suggested that no difference be made between various species, if Apocynum and its preparations were recognized in the N. F. VI, that the one-hour frog method should be recommended for bioassay, and that the potency requirement established for Apocynum and its preparations require them to have twice the strength of digitalis and the corresponding digitalis preparations (3).

Botanical studies have been made on various species of *Apocynum* by Holm (4), Ballard, (5), Gray, Greene, Fernald, Woodson and others, the most extensive treatise on the taxonomy of the group being that of R. E. Woodson, Jr. (6).

The chemistry of Apocynum is not completely worked out. In 1883, O. Schmiedeberg (7) obtained two products in an amorphous state from *A. cannabinum* which he designated as apocynin and apocynein, the latter regarded as a saponin. In 1908, Finnemore (8) found apocynin identi-

cal with acetovanillone. In 1909 the same worker (9) found a bitter active principle called "cynotoxin ($C_{20}H_{28}O_6$ m. p. 165°)" in *Apocynum cannabinum*. The following year Moore (10) discovered a similar principle in *A. androsæmifolium* called "apocynamarin ($C_{14}H_{18}O_3.H_2O$ m. p. 170–175°)." Whether or not these substances are chemically identical or related is unknown.

The researches of Dale and Laidlaw (11) have, however, showed that they are at least alike in their physiological effects and that they belong to the Digitalis group of cardiac stimulants. Impens (12) believes the active principle to be cymarin (m. p. 135–140°) which he states is the same for both species. Windaus and Hermanns (*Apoth. Ztg.*, 1915, 337) state that *anhydrous cymarin* has the formula of $C_{30}H_{44}O_9$.

Materials.—The materials investigated consisted of botanically authenticated plants of *Apocynum cannabinum* collected along the shores of Lake Massapoag, Sharon, Mass., of a variety of *A. cannabinum* collected at Chapel Hill, N. C., and of *Apocynum androsæmifolium* collected in a hilly woodland at New Ipswitch, N. H., also numerous samples of commercial drug collected from scattered sections of the United States. Fluidextracts were prepared from the rhizomes and roots of each of these species according to the National Formulary specifications.

Plants.—*Apocynum cannabinum* L. or Canadian Hemp was found to differ from *Apocynum androsæmifolium* L. or Spreading Dogbane chiefly in the following particulars:

	Apocynum cannabinum.	*Apocynum androsæmifolium.*
Usual occurrence	In gravelly and sandy soil, mostly along streams	In open woodlands and dry thickets
Stems	Erect to ascending with opposite or sub-opposite branches	Dichotomously branched, the branches chiefly alternate
Inflorescences	Dense cymes	Loose and spreading cymes
Flowers	Greenish to greenish white, erect	Pink or pinkish white, mostly nodding
Corolla	Bell-shaped with 5 ascending lobes	Bell-shaped with 5 recurved lobes
Calyx	Tubular, its lobes about as long as the corolla tube	Tubular, about half as long as corolla.
Leaves	Lance-ovate to ovate-oblong and lanceolate. Mostly narrower	Ovate to ovate-oblong or ovate-lanceolate. Mostly broader

The underground system of both species consists of a horizontal, stout, woody, gemmiferous root often mistaken for a rhizome which bears slender, branching, fibrous rootlets. From this gemmiferous root lateral buds arise at intervals which form vertical rhizomes. The rhizomes also produce slender, branching, fibrous rootlets. As shown by Holm (4) the rhizomes are typical root shoots. The aerial stems are continuous with these. As shown by Woodson (6) the young gemmiferous roots are produced laterally from the vertical rhizomes.

Crude Drug.—Recent lots of crude drug obtained during 1938 from scattered sections of the U. S. A., were found to consist mainly of mixtures of both *Apocynum cannabinum* and *A. androsæmifolium*. One sample consisted entirely of *A. androsæmifolium*.

Microscopical examination of botanically authenticated rhizomes and roots of both pure species in serial sections, cut at numerous levels from apex to posterior end of rhizome and from one extremity to the other of gemmiferous roots, showed that stone cells are absent in the rhizomes and roots of *A. cannabinum* and always present in those organs of *A. androsæmifolium*. Both rhizome and gemmiferous roots contain tubular latex cells and also resin cells with a yellow resin content. These were found in cortex and phloem of the gemmiferous roots and in the cortex, phloem, pith and pericycle of the rhizomes of both species. Pericyclic fibers were found in the upper portion of the rhizome and in the aerial stems of both species. The cork cells in both species showed slight lignification. Intraxylary phloem was found in the form of an interrupted circle of sieve and phloem parenchyma strands separated from the xylem by a layer or two of thin-walled cells. The starch grains in the subterranean organs of both species were numerous, up to 20μ in diameter in *A. cannabinum* and 21μ in diameter in *A. androsæmifolium*. The latex cells of the former species were up to 240μ in diameter and in the latter, up to 208μ in diameter. The wood of both species exhibited numerous porous wood fibers associated with tracheæ having multiseriate circular to somewhat hexagonal, closely set bordered pits. The tracheæ were found to be more numerous in sections of the gemmiferous roots than in those of the rhizomes of both species of

similar age. The maximum width of the tracheæ in *A. cannabinum* was 196μ and in *A. androsæmifolium* 180μ.

One of the several *Apocynum cannabinum* plants recently collected for us by Professor Totten at Chapel Hill, N. C. possessed stone cells in the upper portion of the rhizome, but none were found in the lower portion of that organ nor in the attached gemmiferous root. There is a possibility of this plant representing a variety of the species and that *A. cannabinum* may be variable in respect to the stone cell character in the rhizome. Further work is contemplated on this question.

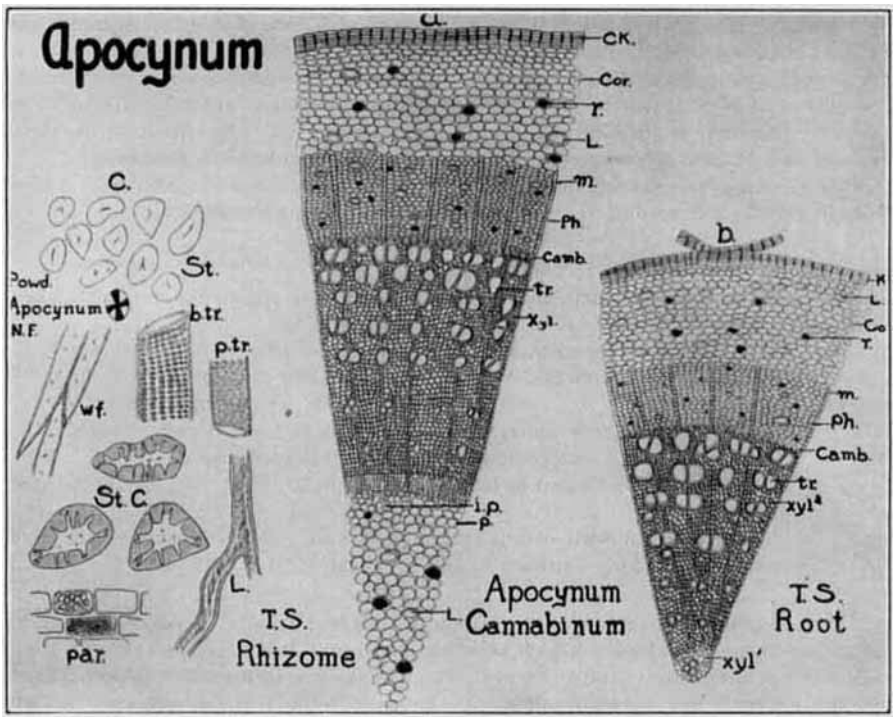

Fig. 4.—Apocynum cannabinum. *a.*, Cross section of rhizome. *b.*, Cross section of root. *c.*, Powdered drug. *ck.* and *k.*, cork; *cor.*, cortex; *r.*, resin cells; *l.*, latex cells; *ph.*, phloem; *i. p.*, internal phloem; *camb.*, cambium; *tr.*, tracheae; *xyl.*, xylem; *xyl.*², secondary xylem; *xyl.*¹, protoxylem; *p.*, pith. *st.*, starch grains. The lowermost grain showing polarization cross under polarized light; wood fibers; *st. c.*, stone cells; *par.*, parenchyma; *L.*, latex cell; *b. tr.*, trachea, with bordered pores; *p. tr.*, pitted trachea.

Ash.—Duplicate determinations of acid-insoluble ash were made on 5 samples of commercial Apocynum according to the U. S. P. XI method, with the following results:

No. 1	Sample A	0.66 %	
	Sample B	0.71 %	Average 0.685%
No. 2	Sample A	2.18 %	
	Sample B	2.07 %	Average 2.12 %
No. 3	Sample A	2.007%	
	Sample B	2.03 %	Average 2.018%
No. 4	Sample A	1.84 %	
	Sample B	1.76 %	Average 1.8 %
No. 5	Sample A	1.28 %	
	Sample B	1.21 %	Average 1.24 %

Average of 5 samples—1.57%

Stem Bases.—Determinations were made on 5 samples of commercial drug for stem bases with the following results:

No. 1	Total drug	79.1500 Gm.	
	Stem bases	2.9260 Gm.	Result 3.7%
No. 2	Total drug	94.6254 Gm.	
	Stem bases	5.4020 Gm.	Result 5.7%
No. 3	Total drug	107.5530 Gm.	
	Stem bases	3.6020 Gm.	Result 3.3%
No. 4	Total drug	98.1084 Gm.	
	Stem bases	2.5796 Gm.	Result 2.6%
No. 5	Total drug	85.8100 Gm.	
	Stem bases	2.8900 Gm.	Result 3.4%

Average—3.74% (Present N. F. standard not more than 5% of stem bases)

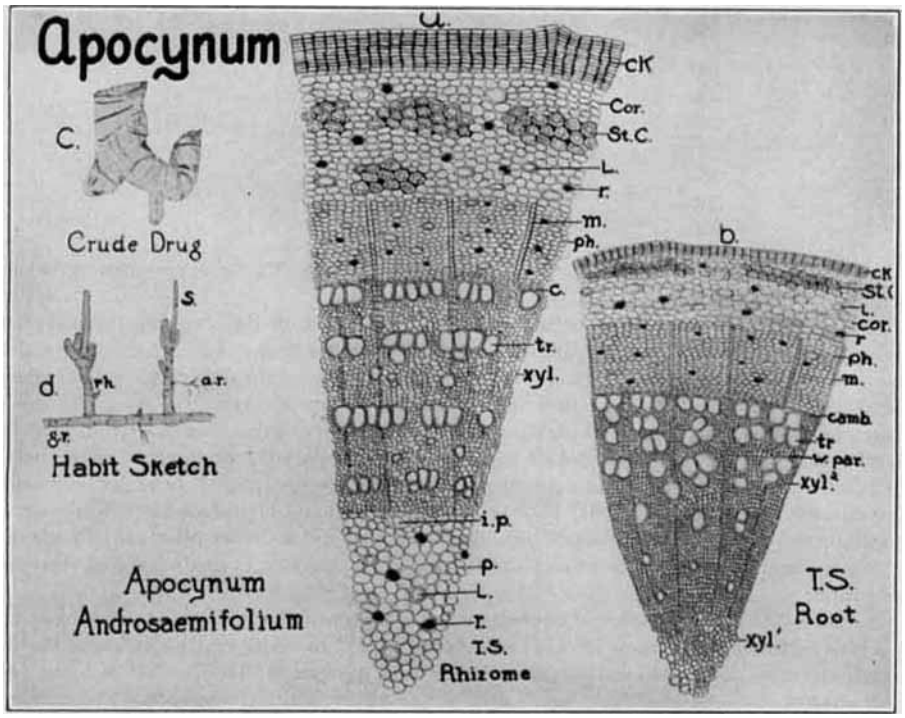

Fig. 5.—Apocynum androsaemifolium. *a*, cross section of rhizome. *b.*, cross section of root; *c.*, piece of crude drug. *d.*, habit sketch of gemmiferous root (*g. r.*) and rhizome (*rh.*) system, the aerial stem (*s.*) being a continuation of the vertical rhizome; *a. r.*, fibrous roots which spring from rhizome and horizontal gemmiferous root; *ck.*, cork; *cor.*, cortex; *St. c.*, stone cells; *L.*, latex cells; *r.*, resin cells; *ph.*, phloem; *c.*, cambium; *m.*, medullary ray; *xyl.*, xylem; *tr.*, trachea; *i. p.*, internal phloem; *pi.*, pith; *w. par.*, wood parenchyma; *xyl.*^{1}, protoxylem; *xyl.*^{2}, secondary xylem.

Determination of Relative Potency of *A. cannabinum* and *A. androsæmifolium*—Fluidextracts were made up according to N. F. specifications from pharmacognostically identified segments of rhizomes and roots of both *A. cannabinum* and *A. androsæmifolium*. The One-Hour Frog Method was adhered to in determining the relative potency of each preparation.

The following tables represent a summary of the work:

TABLE I.—APOCYNUM CANNABINUM.

Dosage in Mg. per Kg.	Dilution of Fluidextract.	Result Systolic Standstill 1 Hour.
70	1/200	0/4
75	1/200	0/4
80	1/200	2/4
85	1/200	4/4
90	1/200	4/4
100	1/100	4/4
150	1/100	4/4
200	1/100	4/4
300	1/100	4/4

The M. S. D. of *A. cannabinum* on frogs is 85 mg./Kg. or approximately 6 times the potency of Digitalis.

TABLE II.—APOCYNUM ANDROSÆMIFOLIUM.

Dosage in Mg. per Kg.	Dilution of Fluidextract.	Result Systolic Standstill 1 Hour.
150	1/50	0/4
170	1/50	0/4
190	1/50	0/4
200	1/50	0/4
210	1/50	0/4
220	1/50	1/4
225	1/50	2/4
230	1/50	4/4
240	1/50	4/4

The M. S. D. of *A. androsæmifolium* on frogs is 225 mg./Kg. or approximately twice the potency of Digitalis.

The difference in potency between the two species, no doubt, explains the inconsistent results which have been obtained from the use of this drug in the past, since the commercial drug consists of varying amounts of these species. A way to overcome the difficulty would be to introduce a standard of assay into the next revision of the National Formulary, using the one-hour frog method for bioassay and requiring Apocynum to have twice the potency of Digitalis. A fluidextract made from *A. androsæmifolium* would need little adjusting to conform to the potency requirement while that made from a mixture of the two species or from *A. cannabinum* would require dilution. It is our belief that both of these species should be included as official sources of Apocynum since they possess the same physiological action and are more potent than Digitalis.

As a result of our work, we recommend the following changes in the present Apocynum monograph:

In line 3 the definition should be changed from "*Apocynum* consists of the dried rhizome and roots of *Apocynum cannabinum* Linné (Fam. *Apocynaceæ*)" to "Apocynum consists of the dried rhizome and roots of *Apocynum cannabinum* Linné or of *Apocynum androsæmifolium* Linné (Fam. *Apocynaceæ*).

DESCRIPTION AND PHYSICAL PROPERTIES.

Unground Apocynum.—Line 1. "Cylindrical, somewhat branched, of varying length, 3 to 10 mm. thick" should be changed to read "cylindrical, sometimes branched segments of the rhizome and roots up to 11.5 cm. in length and up to 1.5 cm. in diameter."

Line 4. "Wood radiate and with large tracheæ" should be changed to read "wood, lemon-yellow, porous, slightly radiate and possessing large tracheæ."

Line 5. "Odor slight" should be added.

Structure.—Line 1. "Cork of 5–15 layers of tangentially-elongated cells with slightly lignified, thickened walls" should be added.

Lines 1–4. "Cortex chiefly parenchyma cells" should be changed to "cortex, a somewhat narrow zone of starch-bearing parenchyma and numerous thin-walled, latex-bearing cells. *A. androsæmifolium* has groups of stone cells in the cortex which are usually absent in *A. cannabinum*." "Phlœm made up of a wide zone of narrow medullary rays which are 1–2 cells wide

rarely 3 cells wide" should be added. "Xylem, a broad radiating porous region, composed of narrow wood wedges made up of numerous large tracheæ" should be added. "Rhizomes with intraxylary phlœm strands" should be added.

Powdered Apocynum.—Line 3. "Polarization crosses distinct" should be added.

Line 3. "Numerous fragments of strongly lignified wood fibers, the latter associated with tracheæ having bordered pores or spiral thickenings," should read "numerous slender lignified, porous wood fibers, associated with tracheæ having simple pits or ellipitical bordered pores."

Line 6. "Stone cells few or absent" should read "stone cells isodiametric or elongated, having stongly lignified, thick walls and branching pore canals."

The following bioassay standard should be added: "Determine the potency of Apocynum in terms of U. S. P. digitalis units as directed for Digitalis in the U. S. Pharmacopœia XI, page 136.

The drug should be additionally described as "giving rise to occasional short rootlets or root scars or purplish buds of aerial stems, and short stem bases with a thin fibrous bark and a hollow center."

The suggestion is made that the dose of three grains be changed to one grain, since apocynum has a higher potency than Digitalis but now is given twice the dosage of Digitalis Pulverata.

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GELATIN AS AN EMULSIFYING AGENT IN LINIMENTUM TEREBINTHINÆ ACETICUM.*

BY FREDERICK GRILL[1] AND NORMAN NOBACH.[1]

Linimentum Terebinthinæ Aceticum represents an emulsion of a volatile oil and water stabilized by fresh egg. Little information could be obtained from the literature reviewed regarding this National Formulary preparation, especially as to the use of different emulsifying agents. Some investigators report a change in the proportion of albumin and yolk of the egg or a modification of the official formula by the addition of a saponin (1)–(2). It has been pointed out by Tice (3) that gelatin from an acid-treated precursor having an isoelectric point at p_H 8 requires a p_H of approximately 3 to effectively stabilize an emulsion. Serrallach and co-workers (4) in determining the film strength of emulsifier films at liquid-liquid interfaces show that comparatively strong films are formed rather rapidly, and continue to increase in strength, at the liquid-liquid interfaces when aqueous solutions of gelatin are added to the fixed oils, castor oil, cod liver oil, olive oil and mineral oil.

Considering the foregoing statements, it was thought that gelatin might prove of value in making Linimentum Terebinthinæ Aceticum because the p_H of the

* Presented before the Section on Practical Pharmacy and Dispensing, A. Ph. A., Minneapolis meeting, 1938.

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