

lized from methanol-water (three times); 9.5 Gm. (65%), m.p. 113-114°.

Anal.—Calcd. for $C_{16}H_{16}O_5$: C, 66.65; H, 5.61. Found: C, 66.50; H, 5.80.

6 - (2 - Acetoxy - 1,4 - naphthoquinon - 3 - yl) - hexanoic Acid (XXI).—The boron trifluoride procedure described for IX and XI (11) was applied to 15 Gm. of XIX in 150 ml. of glacial acetic acid. After 2 hours at room temperature, water was carefully added and the yellow crystalline precipitate was recrystallized from methanol-water; 9.0 Gm. (53%), m.p. 85-86°.

Anal.—Calcd. for $C_{18}H_{18}O_6$: C, 65.50; H, 5.50. Found: C, 65.58; H, 5.31.

Complexes with 1-Naphthylisocyanate (XV): XVI, XVII, XVIII, XX.—The following procedure (XV + V) was also applied to XV and IIIc, XII, and XIX. A solution of 2 Gm. of V in 10 ml. of absolute chloroform was prepared by gentle warming, and 2-3 ml. of XV was added dropwise, whereupon yellow crystals immediately precipitated. Upon cooling the product was filtered off and an infrared spectrum taken. It was then washed 10 times with absolute ether and dried over phosphorus pentoxide. No change in the intensity of the 2260 cm^{-1} band in the spectrum was observed after washing; 1.8 Gm. (77%), m.p. 106°.

The same complex was obtained by refluxing a chloroform solution of V and XV for 5 hours. Neither the lawsone nor the primary alcohol groups in the side chain were affected.

When the complex was dissolved in absolute ethanol, the ethyl urethane was readily formed,

and the quinone moiety could be recovered by addition of ether to the reaction mixture.

Analytical data for the complexes are collected in Table III.

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Comparative Chromatographic Identification of the Alkaloids of Ten *Aquilegia* Species

By CHARLES L. WINEK, JACK L. BEAL, and MICHAEL P. CAVA

The results of a comparative study of the alkaloid constituents in the roots of ten species of *Aquilegia* are reported. Magnoflorine was present in all of the species, while in eight of the species berberine and aquilegenine were also present. A fourth alkaloid, unidentified, is believed to be present in the roots of *A. canadensis*.

THE GENUS *Aquilegia* is classified botanically among the *Ranunculaceae*. The *Ranunculaceae*, like the closely related (1) *Berberidaceae*, constitute a family among which a number of alkaloid-containing genera are found (2, 3). In this paper the results of a comparative study of the alkaloid constituents of ten species of *Aquilegia* are reported.

A literature search revealed a paucity of phytochemical information concerning the genus *Aquilegia*. Indeed, the only report of a

constituent analysis of an *Aquilegia* species dates from 1897, when De Rochebrune (4) described the isolation of an alkaloid, which he called aquilegine, from the seed of *A. vulgaris*.

Munz recognizes 67 species of *Aquilegia* (5) distributed throughout the world. Of these, there are approximately 24 species in North America.

It is readily apparent when examining monographs on *Aquilegia* (5, 6) that most species have several synonymous names which would lead one to believe that a larger number of species must have been considered to exist prior to the thorough study of the genus. *Aquilegias* are found throughout the entire United States and were considered at one time for the title of

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TABLE I.—SPECIES OF *Aquilegia* SCREENED

Species	Source	No. Plants	Av. Dried Wt. per Root, Gm.	Methanol Extractable Material in Dried Roots %
<i>A. canadensis</i>	Gardens of the Blue Ridge, Ashford, N. C.	50	8.34	18
<i>A. vulgaris erecta edelweisse</i>	American Perennial Gardens, Garden City, Mich.	25	7.32	36
<i>A. skinneri</i>	American Perennial Gardens, Garden City, Mich.	25	3.64	21
<i>A. oxysepala</i>	American Perennial Gardens, Garden City, Mich.	15	1.67	10
<i>A. Jonesii</i>	American Perennial Gardens, Garden City, Mich.	15	0.20	..
<i>A. canadensis nana</i>	American Perennial Gardens, Garden City, Mich.	15	18.2	40
<i>A. chrysantha</i>	Springbrook Gardens, Mentor, Ohio	50	9.84	44
<i>A. chrysantha alba</i>	Springbrook Gardens, Mentor, Ohio	50	6.56	25
<i>A. McKana hybrid</i>	Springbrook Gardens, Mentor, Ohio	50	10.4	40
<i>A. caerulea</i>	Wayside Gardens, Mentor, Ohio	100	9.83	40

national flower (7). As with many plants, *Aquilegia* has acquired various common names. In many sections of the United States it is known incorrectly as honeysuckle (8, 9). Probably the most widespread common name of *Aquilegia* is columbine. Two references to the medicinal use of the genus *Aquilegia* have been encountered. Culpeper (10) wrote in 1814 that the leaves of columbine were commonly used in lotions for the treatment of sore mouths and throats, and that seeds of the plant were used in treatment of

jaundice and as an oxytocic. He reported also that Spaniards used the root to treat kidney stones. Felter and Lloyd reported (11) in 1884 that columbine possessed antiscorbutic, diuretic, and diaphoretic properties. They also wrote that Linnaeus regarded the plant as toxic to children.

The alkaloids magnoflorine, berberine, and aquilegine have been isolated from *Aquilegia hybrida* var. Scott-Elliott (16).

Extracts of *A. hybrida* var. Scott-Elliott and authentic samples of magnoflorine chloride and

TABLE II.—SUMMARY OF R_f VALUES OF *Aquilegia* SPECIES QUATERNARY FRACTION

Species	Solvent System	No. of Alkaloidal ^a Spots	Fluorescence, U.V.	R_f
<i>A. hybrida</i> , Scott-Elliott	PAW ^c	1	Blue (magnoflorine)	0.54
	BAW ^d	1	Blue (magnoflorine)	0.55
	PEW ^e	1	Blue (magnoflorine)	0.26
<i>A. canadensis</i>	PAW	2	Blue	0.55
			Nonfluorescent	0.83
	BAW	2	Blue	0.55
			Nonfluorescent	0.85
	PEW	2	Blue	0.26
		Nonfluorescent	0.63	
<i>A. canadensis nana</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. vulgaris erecta edelweisse</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. skinneri</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. oxysepala</i> ^b	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. Jonesii</i> ^b	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. chrysantha</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. chrysantha alba</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. McKana hybrid</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26
<i>A. caerulea</i>	PAW	1	Blue	0.55
	BAW	1	Blue	0.55
	PEW	1	Blue	0.26

^a Stained with Dragendorff's reagent. ^b Represents total extractive. ^c Propanol, ammonium hydroxide, water; 2:1:1. Butanol, acetic acid, water; 5:1:4 (top phase, ascending). ^d Pyridine, ethyl acetate, water; 310:750:165.

TABLE III.—SUMMARY OF R_f VALUES OF *Aquilegia* SPECIES TREATED CHLOROFORM FRACTION

Species	Solvent System	No. of Alkaloidal ^a Spots	Fluorescence, U.V.	R_f
<i>A. hybrida</i> Scott-Elliott	PAW ^b	2	Yellow (berberine) Nonfluorescent ^e	0.58 0.85
	BAW ^c	2	Yellow (berberine) Nonfluorescent ^e	0.41 0.84
	PEW ^d	2	Yellow (berberine) Nonfluorescent ^e	0.35 0.54
<i>A. McKana</i> hybrid	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. chrysantha</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. chrysantha alba</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. canadensis</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. vulgaris erecta edelweisse</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. skinneri</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. canadensis nana</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54
<i>A. caerulea</i>	PAW	2	Yellow Nonfluorescent	0.58 0.85
	BAW	2	Yellow Nonfluorescent	0.41 0.84
	PEW	2	Yellow Nonfluorescent	0.35 0.54

^a Stained with Dragendorff's reagent. ^b Propanol, ammonium hydroxide, water; 2:1:1. ^c Butanol, acetic acid, water; 5:1:4 (top phase, ascending). ^d Pyridine, ethyl acetate, water; 310:750:165. ^e Identical with the R_f value of the non-fluorescent compound, aquilegine, isolated from *A. hybrida* var. Scott-Elliott.

berberine chloride were used as reference samples in this study, the object of which was the screening of ten additional species of *Aquilegia* for the presence or absence of the alkaloids present in *A. hybrida* var. Scott-Elliott.

EXPERIMENTAL

Procurement of Plant Material and Extraction of Roots.—Whole plants of the *Aquilegia* species listed in Table I were obtained as indicated by the source of supply.¹ The plants were dried in an oven at 60°

¹ A plant of each species is being grown to serve as a herbarium specimen.

and the aerial portions separated from the roots. The dried roots were ground to a coarse powder in a Wiley mill. The number of plants obtained and the resulting average dry weight per root is reported for each species in Table I. This information is given to afford an appreciation of the variance in plant size of the species under investigation. One-hundred grams of each species (except for *A. Jonesii*, *A. skinneri*, and *A. oxysepala* which yielded lower weights as indicated in Table I) were extracted in a Soxhlet apparatus with methanol. Extraction was carried to exhaustion as indicated by a negative Valser's test. The extracts were concentrated to

dryness and the weights recorded (Table I). Each total extract was dissolved in dilute acid (aqueous hydrochloric, pH 2), made alkaline with ammonium hydroxide solution, and extracted with chloroform in a rotary film extractor.³ This procedure yielded two fractions: an aqueous alkaline mother liquor (quaternary fraction) and a chloroform fraction.

Paper Chromatography.—The descending technique was utilized (except with one solvent system where the ascending technique was used) as indicated in Tables II and III. Sheets of Whatman No. 1 paper were employed. Extracts were chromatographed simultaneously with reference extracts and authentic alkaloids. The spotted paper sheets were equilibrated for several hours prior to development. Developed, air-dried chromatograms were viewed under long wave ultraviolet light to mark fluorescent spots and were then sprayed with Dragendorff's reagent (13) to reveal positive alkaloidal spots. All R_f values were calculated from measurements taken at the center of stained spots.

Quaternary Fractions.—The aqueous alkaline mother liquor from each species was made acid (pH 4) with 10% HCl, and the quaternary bases were precipitated with ammonium reineckate after the procedure of Panouse (14) as modified by Hogg (15). The alkaloidal reineckates were converted to the corresponding alkaloidal chlorides *via* the method of Kampfhammer (16). The resulting alkaloidal chlorides were dissolved in alcohol and chromatographed simultaneously with the corresponding fraction from *Aquilegia hybrida*, var. Scott-Elliott and with an authentic sample of magnoflorine chloride. The results are indicated in Table II.

Chloroform Fractions.—The chloroform fraction from each species was concentrated and chromatographed along with the corresponding fraction from the Scott-Elliott variety. However, because of extraneous matter, the chloroform fractions yielded brown areas on developed chromatograms which made positive alkaloidal spots difficult to discern with Dragendorff's reagent. It was then found that the alkaloids from this fraction could be extracted in purer form from an acid solution with chloroform. Therefore, each chloroform fraction was concentrated to dryness, and the residue was dissolved in dilute hydrochloric acid (10%) and again extracted with chloroform in a rotary film extractor. The resulting clear brown chloroform fractions were then chromatographed simultaneously with a known sample of berberine chloride and the corresponding chloroform fraction from *A. hybrida* var. Scott-Elliott as references. The results are listed in Table III. Because of a limited supply of plant material, a chloroform fraction was not prepared from *A. oxysepala* and *A. Jonesii*.

DISCUSSION

All ten species of *Aquilegia* examined contained alkaloids in the roots. On the basis of paper

chromatographic data obtained with three solvent systems (Tables II and III), a total of only four different alkaloids appeared to be present in the entire group. Three of these compounds were chromatographically identical with the three alkaloids isolated from *Aquilegia hybrida* var. Scott-Elliott—namely, berberine, magnoflorine, and aquilegine (12).

Magnoflorine was present in every species screened in this study. In the case of three species (*A. Skinneri*, *A. McKana*, and *A. canadensis*), its identity was confirmed by the actual isolation of crystalline magnoflorine chloride (15). In the case of two species (*A. Jonesii* and *A. oxysepala*) magnoflorine was the sole alkaloid detected; however, the apparent absence of other alkaloids may be caused by the very limited amount of plant material available in these two instances.

All species investigated (except *A. Jonesii* and *A. oxysepala*) appeared to contain berberine. Since the completion of this work, however, we have been forced to the conclusion that berberine and the closely related palmatine cannot be distinguished satisfactorily by paper chromatography (17). In view of this fact, the possibility that palmatine may occur in *Aquilegia* species cannot be discounted at this time.

Every species containing berberine also contained a third alkaloid apparently identical with aquilegine, a new crystalline quaternary alkaloid found in *A. hybrida* var. Scott-Elliott (12). In addition, a fourth alkaloid, not yet characterized, was detected only in *A. canadensis*. Both the nature of this fourth alkaloid and the constitution of aquilegine are under continued investigation.

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³ Rinco liquid-liquid extractor, Scientific Glass Apparatus Co.