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Ranunculin: The Precursor of the Vesicant Substance of the Buttercup

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Many of the plants belonging to the natural order Ranunculaceae have the property of producing erythema and blistering the skin. From these plants a terpene-like substance can be obtained, which has long been known under the name of anemonin (I). Anemonin, however, has no vesicant properties, and is derived from the active substance by polymerization. The constitution of the active substance known as protoanemonin (II) has been elucidated by Yasuhiko & Fujita (1922) and Kipping (1935). This was shown to be the lactone of γ-hydroxyvinylacrylic acid (III) and is an oil, volatile in steam and very soluble in water. The pure substance or its solution in water polymerizes in the course of a few days to the insoluble crystalline product anemonin. The distribution of protoanemonin among a series of *Ranunculus* spp. was examined quantitatively by Shearer (1938).

The present investigation was undertaken with the object of finding how the protoanemonin was both stable and harmless within the plant itself. A further object was to obtain a readily available supply of protoanemonin for examination of its action on enzymes, so that the properties of this very simple natural vesicant could be compared with those of the well known vesicants.

It has been found that when the tissues are crushed the protoanemonin is liberated enzymically from a glucosidic precursor in the plant. The glucoside itself has been isolated as a crystalline substance very soluble in water, and, unlike protoanemonin itself, is stable both as a solid and in aqueous solution. This substance, for which structure (IV) is suggested, we propose to call ranunculin, as it has been obtained from several species of *Ranunculus*. Ranunculin, while very stable in acid solution, is rapidly broken down in alkaline solution with the liberation of glucose. The protoanemonin thus formed is converted into acetylacrylic acid (V). Ranunculin, on distillation with aqueous sodium acetate, gives a nearly quantitative yield of protoanemonin and is thus a convenient stable source of the latter.

The ease of production of protoanemonin (II) suggests that in the glucoside the sugar residue is attached to a substance closely related to the lactone. Protoanemonin itself could not be regarded, however, as the aglucone because it has no free hydroxyl group. The most natural assumption would be that the glycoside is that of the enol form of acetylacrylic acid. However, as neither the glycoside nor the tetra-acetyl derivative shows the presence of a free carboxyl group, this assumption could not be true. The rapid liberation of the glucose by alkali and the relative stability of the glucoside in acid solution would point to its being a glucose ester of acetylacrylic acid. This, however, will not easily explain the ready formation of the lactone by boiling with sodium acetate solution or as the initial product of

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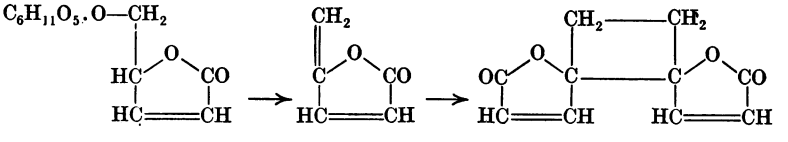
dilute alkali treatment in the cold. If it is assumed that the lactone ring already exists in the glucoside then a structure of the type (IV) results. This hypothetical structure explains the properties so far observed. It is seen that if we adopt this structure liberation of the anhydroaglucone, protoanemonin, will occur without the addition of water, which is an unusual case of the breakdown of a glycoside being analogous to the glycoside picrocrocin described by Kuhn & Winterstein (1933). The properties of the glucoside are such that it would accompany sugars in the usual methods of extracting plant tissues for estimating carbohydrates. Further, it would break down to give glucose during a method of copper reduction. As, in some cases, the substance is present as 1 % of the fresh weight of the plant, it would make it appear that the plant had a high level of hexose, unless the presence of this type of glucoside was suspected.



Ranunculin (IV) (provisional structure) Protoanemonin (II) Anemonin (I)

β-Acetylacrylic acid (V) γ-Hydroxyvinylacrylic acid (III)

EXPERIMENTAL

The formation of protoanemonin from the plant

The fresh plant of *Ranunculus bulbosus* on being bruised in a mortar develops a strong smell of protoanemonin in the course of 3–5 min.; the effect is not instantaneous. The fresh plant bruised with dilute acid in a mortar (pH 2, to destroy all enzymes) does not develop protoanemonin. If the fluid extract is now removed and boiled with sodium acetate, protoanemonin is volatilized with the steam.

It is therefore concluded that protoanemonin is liberated by enzyme action from a precursor in the plant, and that the precursor is decomposed by heating with sodium acetate.

Isolation of the precursor from the plant

The fresh plants were ground in lots of 180 g. with 200 ml. water containing either 5 ml. conc. HNO_3, or 8 ml. conc. HCl. The pulp was pressed out through a cloth and the fluid spun on a centrifuge. The brownish turbid fluid was then filtered through kieselguhr to remove suspended matter. The clear brownish filtrate was then treated with charcoal (3–6 g./180 g. fresh plant) for 20 min. at room temperature. The charcoal was British Drug Houses 'activated', previously boiled in a small quantity of distilled water to expel the air. After this treatment the solution was pale straw-yellow.

The nearly colourless fluid was then treated in a similar way with a larger quantity (up to 20 g./180 g. plant) of charcoal, which now adsorbed the precursor. The charcoal was filtered off on large Büchner funnels on papers covered with a thick layer of kieselguhr. The charcoal was carefully washed with distilled water to remove excess of acid and unadsorbed matter, and the precursor was eluted from the charcoal by slowly sucking 50 % aqueous ethanol through it.

The filtrate was evaporated to a thin syrup under reduced pressure, washed out of the vessel with a small quantity of water and treated with 2 vol. of methanol. A precipitate containing pectin was filtered off and the clear filtrate evaporated down *in vacuo* and finally taken down (in a dish) to a thick syrup, in an air current on a warm bath. Towards the end of the operation the syrup was seeded and stirred until it became a semi-solid mass of crystals. After cooling, small quantities of methanol were added with thorough mixing, until after 20 min. the substance had all crystallized out. It was then filtered off and washed with methanol. A colourless, nearly pure, preparation was thus obtained. Up to 0·9 g. was obtained for each 10 g. charcoal used. Recrystallization was effected by dissolving in warm methanol (12 parts), filtering, and treating as in the isolation of the crude substance.

The melting points of the crude products were usually in the neighbourhood of 140°; in a few cases the products sintered at 135°. The substance was obtained from various species over a period in the early summer of 1942. The identity of the substance from the different species was established by seeding and by mixed melting points. The results of the various preparations are shown in Tables 1 and 2. The yields of ranunculin which we obtained correspond with the protoanemonin found by Shearer (1938) from the same species.

Properties of ranunculin

The substance is a colourless solid crystallizing in nearly rectangular plates, sometimes much elongated, of which 1 g. dissolves in 0·7 ml. of water. Large crystals are formed on evaporation of such a solution. It is sparingly soluble in cold

Table 1. *Isolation of glucoside from four* Ranunculus *species*

	Plant		Yield	
Date, 1942	Species	(g.)	(g.)	Percentage of fresh wt. of plant
18 May	*R. bulbosus*	180	0·6	0·33
19 May	*R. bulbosus*	335	3·25	0·97
26 May	*R. bulbosus*	610	5·37	0·88
30 May	*R. bulbosus*	680	5·45	0·80
27 May	*R. acris*	540	1·05	0·19
8 June	*R. acris*	1300	4·70	0·36
8 June	*R. acris*	800	3·42	0·40
3 June	*R. arvensis*	1050	7·7	0·58
16 June	*R. arvensis*	1260	20·55	1·63
24 June	*R. arvensis*	1080	8·4	0·78
6 July	*R. sceleratus*	500	3·05	0·61

Table 2. *Efficiency of charcoal adsorption in the preparation of ranunculin*

Date, 1942	Species	Charcoal used for nearly complete adsorption (g.)	Yield (g.)	M.p. of crude product (°)
26 May	*R. bulbosus*	50	4·52	139–140
30 May	*R. bulbosus*	50	4·35	139
16 June	*R. arvensis*	100	8·70	136–138

methanol and nearly insoluble in cold ethanol. No loss of weight occurs at 100° *in vacuo* over P_2O_5. The *substance* melts at 141–142° giving a colourless liquid; the melting point is markedly depressed by traces of water. Visible decomposition occurs in the melting point tube only at about 205°. (Found: C, 48·1; H, 5·9. $C_{11}H_{16}O_8$ requires C, 47·8; H, 5·8%.) The substance is laevorotatory $[\alpha]_D^{17·5°} = -80·7°$ (0·417 g. in 25 ml. water, $l = 20$ cm., $\alpha = -4·69°$).

Reactions of ranunculin

Action of alkali. If dilute NaOH is added to a concentrated solution of ranunculin the smell of protoanemonin is noted, but this soon becomes inappreciable. The decomposition with alkali was examined polarimetrically both with NaOH and Na_2CO_3. In both cases at room temperature the rotation changed rapidly towards that of a glucose solution treated similarly (Tables 3 and 4). During the reaction with carbonate an amorphous precipitate formed, soluble in alkali, indicating that side reactions occur.

Table 3. *Action of* NaOH *on ranunculin at* 19°

(0·714 g. ranunculin in 25 ml. 0·1 N-NaOH. 0·500 g. glucose in 25 ml. 0·1 N-NaOH. 0·714 g. ranunculin, $C_{11}H_{16}O_8$, contains 0·5 g. glucose.)

Value of α	Ranunculin	Glucose
Initial	−4·69°	+2·10°
10 min.	+1·15°	+1·83°
1 hr.	+1·19°	+1·60°
24 hr.	+1·02°	+0·47°

Table 4. *Action of* K_2CO_3 *on ranunculin at* 19°

(0·714 g. ranunculin in 25 ml. water + 0·2 g. K_2CO_3. 0·500 g. glucose in 25 ml. water + 0·2 g. K_2CO_3.)

Value of α	Ranunculin	Glucose
Initial	−4·69°	+2·1°
10 min.	−0·80°	+2·1°
24 hr.	+1·42°	+2·1°

Titration with alkali. The glucoside was titrated with alkali at 60° using phenolphthalein or alizarin as indicator. The result was in agreement with there being one lactone ring per molecule of glucose in the compound (Table 5).

Table 5. *Titration of glucoside*

	0·1 N-NaOH (ml.)
Calc. for 0·200 g. substance $C_{11}H_{16}O_8$ (mol.wt. 285)	7·20
0·199 g., indicator phenolphthalein, required	7·07
0·199 g., indicator alizarin, required	7·35

Estimation of free COOH *groups.* Ranunculin displaced no CO_2 from bicarbonate buffer, pH 7·4, 37°, in Barcroft manometers. It was therefore concluded that the COOH group is combined as a lactone ring.

Acetyl derivative. Ranunculin (0·5 g.) was well broken up in a mixture of 12 ml. acetic anhydride and 1·0 ml. glacial acetic acid; 10 ml. pure pyridine were added very slowly with cooling. After 2·5 hr. the mixture was poured on to ice, and the pyridine and part of the acetic acid were distilled off *in vacuo*. The crude substance was precipitated by water and the insoluble residue was crystallized first from absolute ethanol and then from very dilute aqueous methanol.

The *acetyl* compound crystallizes in long colourless needles, m.p. 136–137°. It is appreciably soluble in hot water. (Found: C, 51·4; H, 5·4. $C_{19}H_{24}O_{12}$ requires C, 51·4; H, 5·4%.)

Titration of acetyl compound. (*a*) 0·1 g. in 20 ml. water was titrated at 60° with 0·1 N-NaOH, using phenolphthalein as indicator. The fluid became yellow during the titration. (Found: 11·4 ml. Calc. for 4 acetyl and 1 lactone group: 11·3 ml.) (*b*) 0·102 g. in 12 ml. 0·1 M-NaOH was kept at 60°

for 20 min. and then back titrated with 0·104 N-HCl, using phenolphthalein. (Found: 11·82 ml. 0·1 N-NaOH used. Calc. 11·45 ml.)

Estimation of glucose. The glucoside is so rapidly decomposed by alkali that it reduces Fehling's solution like free glucose. It was titrated with Fehling's solution by the method of Lane & Enyon (1923). (Found 63% glucose. Calc. (structure IV) 70%.)

Formation of glucosazone. Ranunculin was boiled with dilute sodium acetate for 5 min. and then treated at 100° for 20 min. with phenylhydrazine and dilute acetic acid. Glucosazone was produced. No glucosazone was given under similar conditions from the glucoside itself, without previous hydrolysis.

Formation of protoanemonin. The glucoside (0·2 g.) was dissolved in 5 ml. water containing 2 g. sodium acetate, and the solution was steam distilled for 25 min. The distillate was then neutralized using phenolphthalein (1·6 ml. 0·1 N-NaOH were required to neutralize free volatile acid). 8 ml. 0·1 N-NaOH were added and the solution kept at 60° for 45 min. Back titration with 0·1 N-HCl showed that 5·9 ml. 0·1N-NaOH were used.

The distillation was continued for a further 30 min. and the distillate neutralized as before. Back titration: 0·55 ml. Total NaOH: 6·45 ml. Calc. for structure IV: 7·2 ml. 0·1 N-NaOH.

Preparation of protoanemonin. Ranunculin (3 g.) was dissolved in 300 ml. 10% sodium acetate. This solution was distilled at atmospheric pressure for 25–30 min., or until no more protoanemonin came over. Any acetic acid in the distillate (about 150 ml.) was neutralized with $NaHCO_3$, and the solution was saturated with NaCl and extracted eight times with ether. The ether extract (about 120 ml.) was shaken with a little more $NaHCO_3$, dried over Na_2SO_4, and the ether removed *in vacuo*. The residue was a liquid having the vesicant properties described for protoanemonin; it very rapidly polymerized to a solid.

SUMMARY

1. The protoanemonin obtained from species of buttercups (*Ranunculus*) is derived from a glucoside.

2. The isolation and some properties of the glucoside are described; a provisional structure is given.

3. The substance seems likely to be present only in Ranunculaceae, and the name ranunculin is proposed for this glucoside which yields protoanemonin.

4. The pure glucoside is a stable preparation which is a convenient source of protoanemonin.

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Suppression of Catalase Activity by Peroxidase and its Substrates

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While studying the darkening of doughs made from low-grade flours, the authors wished to ascertain whether the peroxidase of wheat germ could function in the presence of the catalase also present in germ. A vast amount of work has been done on catalase as well as on peroxidase, but little seems to be known of what happens on addition of hydrogen peroxide to a system showing both catalase and peroxidase activities. Zeile (1934) expresses the opinion that peroxidase activity is inhibited in the presence of catalase. Later in the same article he says that, if catalase is present in small amounts only, peroxidase activity is predominant, whereas large amounts of catalase cause purely oxidative effects by providing molecular oxygen for oxidases. This is how Sumner & Somers (1947) sum up the situation: 'It would appear that peroxidase is of value to plants and animals to bring about the oxidation of certain phenols, but there is little or no experimental evidence to support this. Whether enough hydrogen