EXPERIMENTAL

Radiochemicals. Sodium [1-^{14}C] acetate (sp. act. 59.3 mCi/mM), sodium [2-^{14}C] malonate (17 mCi/mM) and [*n* 9, 10] oleic acid (2.4 Ci/mM) were purchased (Amersham). Counting was carried out [4] on an Intertechnique scintillation counter LS20.

Plant materials and feeding techniques. Seeds of *Vicia faba* L. (cv Aquadulcia claudia) were purchased (Suttons). The seeds were placed in plastic trays between layers of moist tissue paper and left to germinate and imbibe for 3 days. After this period the outer seed coat and the developing stem and root were removed and discarded. *Botrytis cinerea* was cultivated on standard MX media or V8 agar for 20 days. Spore concns were usually in the order of 5×10^6 conidia/ml. Spores were centrifuged and washed with sterile distilled H_2O prior to use.

Prepared cotyledons were placed on moist tissues in sandwich boxes and infected with spores from mature sporulating cultures of *Botrytis cinerea.* The prepared seeds were then allowed to stand for 15 hr during which time the spore suspension soaked into the bean tissue. After this time the labelled substrates were applied as aq. solns, the boxes closed and the seeds incubated at room temp. for 5 days. After this period the necrotic lesions were removed, frozen in liquid N_2 and ground in a pestle and mortar. The finely ground lesions were soaked overnight in Et_2O, filtered, washed with Et_2O, the Et_2O extracts dried, filtered and concd. The conc soln was then subjected to PLC on 20 × 20 cm Si gel plates (Merck GF_{254}) in hexane/Me_2CO (2:1) followed by $CHCl_3$/petrol (2:1). The wyerone (R_f *ca* 0.6) was removed from the plate and eluted with Et_2O. The crude wyerone was diluted with carrier (*ca* 20 mg) and crystallized to constant mp and specific activity. TLC fails to separate wyerone from dihydrowyerone. However, we have previously shown that repeated crystallization of wyerone from cyclohexane removes all traces of the dihydro derivative as evidenced by the disappearance of the M + 2 peak in the MS of wyerone purified in this manner.

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ANEMONIN, PROTOANEMONIN AND RANUNCULIN FROM *KNOWLTONIA CAPENSIS*

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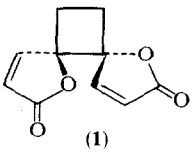
Key Word Index—*Knowltonia capensis*; Ranunculaceae; anemonin; protoanemonin; ranunculin; lactones.

The genus *Knowltonia* (Ranunculaceae), is endemic to South Africa and there are a large number of reported uses of these plants in folk medicine [1]. Prior to this report, no detailed chemical studies had been carried out on members of this genus.

Aqueous and ethanol extracts of fresh *Knowltonia capensis* exhibited significant antibacterial activity against *Staphylococcus aureus*, while extraction of the dried ground plant material according to the preliminary fractionation procedure recommended by the National Cancer Institute [2] gave a chloroform fraction which exhibited significant *in vivo* antileukemic activity in the P388 lymphocytic leukemia test system [3]. Extraction of dried ground plant material with ethanol, followed by partitioning of the extract between ether and aqueous acid and extraction of the neutralized aqueous fraction with chloroform, gave a fraction which accounted for most of the observed antibacterial activity. Chromatography of this fraction on silica gave anemonin (**1**). Steam distillation of fresh plant material and extraction of the steam distillate with chloroform afforded protoanemonin (**2**) [4], which rapidly polymerized to anemonin. Extraction of fresh plant material with dilute HCl according to the procedure of Hill and van Heyningen [5] yielded the stable glucoside, ranunculin (**3**).

Ranunculin has been postulated as the precursor of protoanemonin [5], which has been shown to occur exclusively in members of one tribe of the Ranunculaceae, the Anemoneae and in the genus *Helleborus* [6]. However, in 1972 Carl Tschesche and coworkers found that, whereas extraction of *Ranunculus repens* and *Helleborus foetidus* according to the method of Hill and van Heyningen yielded ranunculin and a new isomer isoranunculin (**4**), when the plant material was carefully extracted with aqueous acetone, no trace of ranunculin could be found and two new glycosides, ranuncoside (**5**) and ranunculoside (**6**) were isolated [7]. These workers therefore concluded that ranunculin is an artifact generated during the acid extraction procedure, and that the genuine precursor of protoanemonin has yet to be isolated.

The aqueous acetone extraction of fresh *Knowltonia capensis*, however, afforded ranunculin as the major component and we were unable to detect the presence



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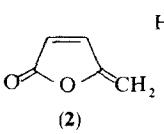

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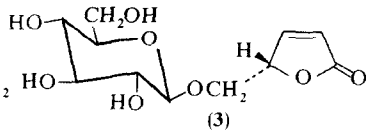

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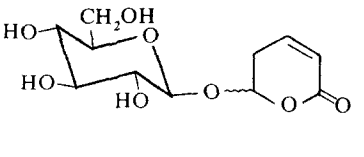

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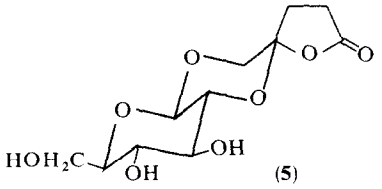

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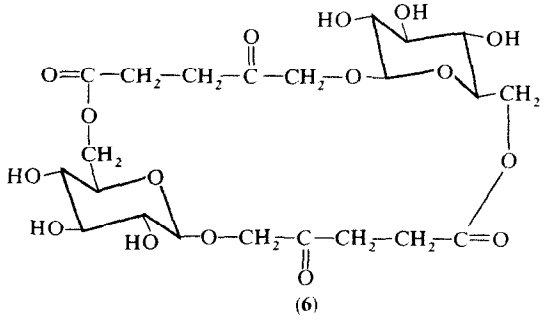

(6)

of ranuncoside and ranunculoside. Furthermore, in the HCl extract of the plant we could find no trace of isoranunculin. It would appear, therefore, that these compounds are not present in this member of the genus *Knowltonia*, and that ranunculin is not an artifact in this particular plant.

EXPERIMENTAL

Plant source. Plants of known taxonomic authenticity were collected in Kirstenbosch National Botanical Gardens, Cape Town. (Representative voucher specimen Esterhuysen 182 in Compton Herbarium, Kirstenbosch National Botanical Gardens, Cape Town.)

Isolation of anemonin (**1**). Dried ground plant material (3 kg) was continuously extracted with EtOH (25 l.) for 6 days. Concn of the extract to 4 l. gave a white crystalline compound (12 g) which was identified as glucose. Evaporation of the remaining alcohol *in vacuo* gave a residue (900 g) which was taken up in H_2O (1.5 l.), acidified with conc HCl and extracted with Et_2O (4 l.). The aq. fraction was neutralized with dil NaOH and extracted with $CHCl_3$ (5 l.) to give 22 g of extract. Dry column chromatography of the extract (10 g) on Si gel using $CHCl_3$ and $CHCl_3$–EtOAc as eluants gave anemonin (3.7 g) which was recrystallized from MeOH to give needles, mp 149–150° (lit. 151–152°).

Me_2CO/H_2O *extraction of plant.* Fresh plant material (300 g) was freeze-dried and then extracted in the cold with Me_2CO–H_2O (1:1, 3 × 500 ml). The Me_2CO was removed under red. pres. and at room temp. and the aq. soln freeze-dried to a thick syrup (5 g) which was chromatographed on Si gel (200 g dry-packed). Elution with 30% MeOH in $CHCl_3$ gave a gum (300 mg) which crystallized on standing and which was shown by NMR to be ranunculin.

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