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ISOLATION OF FUMARIC ACID, SUCCINIC ACID, ASTRAGALIN, ISOQUERCITRIN AND TILIROSIDE FROM *PTERIDIUM AQUILINUM**

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Key Word Index—*Pteridium aquilinum*; Pteris; bracken fern. fumaric acid; succinic acid; astragalin; isoquercitrin; tiliroside.

Bracken fern (*Pteridium aquilinum*) is used as food for humans, and is an etiologic agent for bovine urinary bladder tumors reported in high incidence, in various parts of the world.[1] Administration of this plant to experimental animals produces a wide variety and high incidence of tumors,[2–7] but the responsible chemical carcinogen has not yet been identified. For the last 5 yr, we have been testing the carcinogenic activity of prepared bracken fern fractions.[5] In one of the fractions displaying greatest carcinogenic potency, fumaric acid, succinic acid, astragalin, isoquercitrin and tiliroside have been isolated and characterized.

Astragalin and isoquercitrin have previously been found in bracken fern,[8] and tiliroside in other plants.[9] The structure of tiliroside was reported first as 7-*p*-coumaroylkaempferol-3-glucoside[10] and then revised to kaempferol-3-(*p*-coumaroylglucoside).[11] We treated tiliroside with diazomethane and isolated 5,7,4′-trimethoxykaemperol as the hydrolysis product, thus supporting the latter structure.

EXPERIMENTAL

Plant and plant extract. Bracken fern was collected in June of 1970 from farms in the Province of Bolu, Turkey where the incidence of bovine urinary bladder tumors is high, and dried in the shade, milled and shipped to our laboratory. 1 kg was extracted with hot MeOH for 72 hr. The extract was concentrated under reduced pressure at 36° to a heavy syrup. The syrup was mixed with 1 l. H_2O, the precipitate removed, and the filtrate extracted with $CHCl_3$. The H_2O phase was then extracted with a mixture of *n*-BuOH–2-butanone (1:1). The organic phase was dried, dissolved in 50 ml MeOH and mixed with 200 ml Et_2O. The MeOH–Et_2O extract was dried and stored in a desiccator.

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Purification. The material from 3 kg brackern fern, was dissolved in 10 ml MeOH and separated on Sephadex LH20 (34·5 × 2·1 cm) eluted with 3·5 l. MeOH. Succinic acid (1·23–1·42 l.) and fumaric acid (1·43–1·64 l.) were obtained and recrystallized (H_2O). Eluates 2·06–2·43 l. were concentrated and stored at 4° for 1 week giving tiliroside, recrystallized from aq. MeOH. The filtrate was dried and treated with EtOAc. The extract was separated on 3M paper in *n*-BuOH–HOAc–H_2O (4:1:5) (BAW). Astragalin and isoquercitrin were obtained and purified by rechromatography.

UV spectral analysis. UV analysis was conducted according to the method described by Mabry *et al.*[12]

PC. Qualitative analysis of flavonoids was on Whatman No. 1 using BAW, isoPrOH–HCOOH–H_2O (2:5:5), 15% HOAc, and 2% HCOOH as solvent. Sugars were detected with ammoniacal $AgNO_3$.

Identification. Fumaric acid, m.p. 287° and succinic acid, m.p. 188–189° were identified by IR, MS and m.m.p. with the authentic compounds. Astragalin produced kaempferol and glucose by hydrolysis. MS did not show the molecular ion. However, the aglycone ion at *m/e* 286 was shown. UV spectral analysis verified the structure. Isoquercitrin produced quercetin and glucose by acid hydrolysis. Although MS did not show the parent ion, aglycone ion at *m/e*, 302 was seen. The structure was confirmed by UV spectral analysis. Tiliroside, m.p. 272–273° had an IR and UV spectrum similar to the reported compound.[10] High resolution MS* did not show the molecular ion, but fragments of kaempferol at *m/e*, 286 and *p*-coumaroylkaempferol at *m/e*, 432 were seen. *p*-Coumaroylkaempferol could be formed due to rearrangement. Acid hydrolysis of tiliroside yielded kaempferol, *p*-coumaric acid, glucose, and one unidentified compound which had properties similar to 3-*p*-coumaroylglucose.[11] The tiliroside was methylated with CH_2N_2 for 3 days in the dark. The methylated product had λ_{Max}^{MeOH} 262 and 310 nm. The spectrum was not changed by NaOMe or $AlCl_3$. The aglycone was isolated after acid hydrolysis by PC. MS showed a large peak at *m/e*, 328. UV spectrum showed only the 3-hydroxyl group was free. Both MS and UV spectrums were compatible with the interpretation of the aglycone as 5,7,4′-tri-methoxykaempferol.

* Generously performed by Drs. T. J. Perun and J. M. Price, Abbott Laboratories, North Chicago, IL 60064, U.S.A.

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STRUCTURE OF A NEW TRITERPENE TRIOL FROM *CALENDULA OFFICINALIS* FLOWERS

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In an earlier paper we described the identification and structural determination of a number of mono- and di-hydroxy triterpene alcohols of the oleanane, lupane and ursane types isolated from *Calendula officinalis* flowers.[1] Triterpene triols and tetrols were also shown to be present in this material. Among these compounds were found α-amyrin (Id) and two diols with an α-amyrin skeleton, brein (Ib) and ursadiol (Ic)[2]. We have now isolated a new triterpene triol closely similar to above compounds, for which we suggest the structure of 3,16,21-trihydroxy-12-ursaene (Ia).

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