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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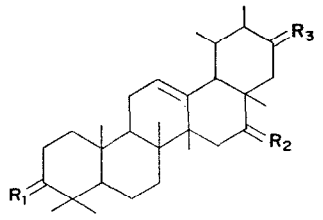

(a) $R_1 = R_2 = R_3 = H,OH$
(b) $R_1 = R_2 = H,OH$: $R_3 = H_2$
(c) $R_1 = R_3 = H,OH$: $R_2 = H_2$
(d) $R_1 = H,OH$: $R_2 = R_3 = H_2$

(I)

The triol (Ia) was characterized as its crystalline triacetate, m.p. 209–213° giving a positive reaction with tetranitromethane. It is not oxidized with SeO_2 as is characteristic for α-amyrin derivatives. The IR spectrum is similar in the 800–1400 cm^{-1} range to that of the acetates of α-amyrin, brein and ursadiol; there is a weak band at 1075 cm^{-1} like brein acetate and the triol also gives a strong band at 1720 cm^{-1} (=C=O) and 1240 cm^{-1} ($\gg$C–O).

The NMR pattern of triol triacetate is closely similar to those of (Ib) and (Ic) diacetates over the range 0·80–1·925 and 4·15–4·80 ppm; there are also signals at (δ, ppm): 4·23, 4·275, 4·40 (3H –CH–OAc), 1·925 (9H –3 × Ac), 1·15–1·00 (6 tert. Me) and 0·80 (2 sec. Me), excluding a β-amyrine type structure. The signals in 4·93–5·58 ppm range characteristic for olefinic proton at C-12 in pentacyclic triterpenes could not be detected in the spectra of the acetates of ursadiol and the new triol (although present in the spectrum of brein diacetate) probably due to its insufficient sharpness, or shifted position when in α-amyrin skeleton.

The MS peaks 524 (0·5), 464 (8·8), 440 (8·5) and lack of peaks 511 and 451 suggest that all three hydroxyl groups are secondary. The intense peaks at 189 (100) and 217 (12·5) proves the presence of double bond in 12, 13 position.[3,4]

Hydrolysis of the triol triacetate yielded a free alcohol m.p. 244–250° and oxidation[5] with CrO_3 yielded a triketone m.p. 280–286°. The ORD and CD spectra of this compound are similar to those of breinone and ursadione. ORD spectrum of the triketone is, however, closer to that of ursadione than to that of breinon, suggesting the greater contribution in this spectrum of the >C=O group in position 21 then in position 16. Breinone: Φ_{260} +8260°, Φ_{273} +8360°, Φ_{305} 0°, Φ_{312} −2706°, Φ_{315} −2618°, Φ_{320} −3170°, Φ_{352} 0°. Ursadione: Φ_{260} +3400°, Φ_{275} +3550°, Φ_{294} 0°, Φ_{310} −2860, Φ_{330} −1500°. Trione: Φ_{260} +2463°, Φ_{272} +2800°, Φ_{294} 0°, Φ_{313} −2260°, Φ_{350} −511°.

The CD amplitude is negative for all three ketones: breine ($\Delta\epsilon$ −2·52), ursadione ($\Delta\epsilon$ −1·48) and the trione ($\Delta\epsilon$ −1·01).

ORD spectra of free alcohols (Ia, Ib, Ic) do not show the Cotton effect in the 260–350 nm. range. In this range, brein exhibits a positive amplitude, ursadiol a negative one[2] and the triol an amplitude approaching zero. Such effects are due probably to the intramolecular compensation of the absorption of OH groups in position 16 and 21 in the triol.

A similar co-occurrence of two triterpenes diols and a related triol in the same plant was recently reported by Kikuchi and Niwa.[6] They found a new triterpene triol of the friedelin type possessing three hydroxyl groups at C-2, C-3, C-16 analogous to the positions occupied by hydroxyl groups in two diols (2, 3 and 3, 16) isolated from the same plant,

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