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CORONOPILIN—ANOTHER MAJOR SESQUITERPENE LACTONE IN *PARTHENIUM HYSTEROPHORUS*

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Key Word Index -*Parthenium hysterophorus*; Compositae; sesquiterpene lactones; coronopilin; parthenin; tetraneurin-A.

INTRODUCTION

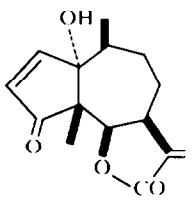
Parthenium hysterophorus L. (Compositae), an aggressive weed of the Americas including the Carribbean, has spread in the last hundred years to Australia, Africa and Asia [1]. In some parts of India it is responsible for a high incidence of allergic contact dermatitis [2]. Parthenin (**1**) was isolated and identified as the major sesquiterpene lactone of this species [3-5] and shown to be the allergen responsible [2]. More recently, other sesquiterpene lactones, tetraneurin-A and the newly described hysterophorin ([6] the structures shown in this reference are incorrect) have been identified as additional allergens from *P. hysterophorus* growing in India. Using a new TLC method for visualization of sesquiterpene lactones [7], we have identified coronopilin (**2**) as the second major sesquiterpene lactone (after parthenin) and tetraneurin-A as a minor sesquiterpene lactone in samples of the plant from both India and Belize, central America.

RESULTS AND DISCUSSION

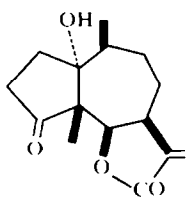
Coronopilin (identified by NMR, mp, R_f and colour on TLC) was found in extracts of dried stems, leaves, flowering heads, trichomes (Belize material) and fresh parts of *P. hysterophorus* grown at UBC. It was also isolated from achenes (Belize) as well as trichome and pollen extracts of Indian samples. The ratio of parthenin to coronopilin is approximately 10:1 (NMR). Coronopilin has been reported from numerous species of *Ambrosia* and *Parthenium* [8, 9] but not *P. hysterophorus* probably because it cannot be easily distinguished from parthenin by conventional methods of TLC analyses for sesquiterpene lactones (i.e. UV light, iodine vapours, or aqueous $KMnO_4$). However, more recently, coronopilin has also been identified in this species by Indian workers (A. Lonkar, personal communication).

The fact that coronopilin occurs in *P. hysterophorus* in appreciable amounts and that it is a strong allergen [10] might explain the reported stronger positive test reaction of patients to acetone extracts of this plant than to parthenin alone [11].

In addition to parthenin and coronopilin, small quantities of tetraneurin-A also occur in shoot and flower extracts but not in the pollen of *P. hysterophorus*. We did not detect ambrosin in any part in spite of its reported occurrence in trichomes [12]. Plant samples from India and Belize are very similar in their sesquiterpene lactone composition but material from various regions in South America (e.g. Argentina) vary considerably in chemical composition [13]. This, therefore, does not support the suggestion that *P. hysterophorus* in India originated from Argentina [14].



1 Parthenin



2 Coronopilin

EXPERIMENTAL

Isolation and identification of coronopilin. Parthenin, isolated from shoots of dried *P. hysterophorus* (collected in Belize) according to the procedure of Rodriguez [15], was found on TLC (Polygram Si gel; $CHCl_3$-Me_2CO, (6:1); vanillin spray reagent [7]) to be a mixture of two compounds. 60 mg of the mixture (in $CHCl_3$) was chromatographed on a Si gel column and eluted with $CHCl_3$ Me_2CO (6:1). Early fractions gave a spot on TLC (R_f = 0.46) which reacted with the vanillin reagent to give a blue colour. The combined fractions yielded fine white crystals, mp 177°, ^{1}H NMR ($CDCl_3$ with TMS): δ 6.29 (*d*, H-13b), 5.61 (*d*, H-13a), 4.95 (*d*, H-6), 1.17 (*s*, C-10-Me), 1.10 (*s*, C-5-Me). The NMR and mp correspond to the reported values of coronopilin [16] and R_f and colour on TLC were identical with those of an authentic sample of coronopilin (a gift from Prof. T. A. Geissman). Later fractions (R_f = 0.44, bluish-green colour with vanillin reagent) yielded pure parthenin, mp 167°, ^{1}H NMR($CDCl_3$ with TMS): δ 7.50 (*d*, H-2), 6.25 (*d*, H-3), 6.33 (*d*, H-13b), 5.62 (*d*, H-13a), 5.02 (*d*, H-6), 1.30 (*s*, C-5-Me), 1.14 (*s*, C-10-Me). The parthenin–coronopilin mixture before chromatography had mp 164° and NMR ($CDCl_3$ with TMS) identical with that given for parthenin [16].

Acknowledgements We thank Dr. E. Rodriguez for samples of tetraneurin-A and ambrosin and the National Science and Engineering Research Council of Canada for financial support.

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ACYCLIC DITERPENES FROM *CROTON KERRII*

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Key Word Index—*Croton kerrii*; Euphorbiaceae; acyclic diterpenes; anti-reserpine ulcer; (*E,E,Z*)-11-hydroxymethyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol; (*E,E,E*)-11-formyl-3,7,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol.

INTRODUCTION

Recently, we reported the structures of 18-hydroxygeranylgeraniol (**1**) [1], an anti-reserpine ulcer substance, and of plaunols A,B,C,D and E [2, 3], anti-Shay ulcer compounds, isolated from a Thai medicinal plant, *Croton sublyratus* Kurz. We have now commenced a chemotaxonomical survey of plants of the genus *Croton* which grow wild in Thailand. From *Croton* sp., various diterpenes belonging to the labdane (or clerodane) [4–10], pimarane [11], tiglane [12], crotofolane [13], and acyclic diterpene [1] have been isolated. We now report the isolation and characterization of two novel acyclic diterpenes from *Croton kerrii* A. Shaw (Euphorbiaceae) [14].

RESULTS AND DISCUSSION

A methanol extract of the leaves of *C. kerrii* was washed with *n*-hexane. The *n*-hexane extract was subjected to silica gel chromatography to yield diterpenes **2a** and **3a**. The diterpene diol (**2a**) had a molecular formula of $C_{20}H_{34}O_2$ by high resolution MS of its bis-trimethylsilylether (Calc. for $C_{26}H_{50}O_2Si_2$: 450, 3349. Found: 450, 3354). The IR, ^{1}H NMR, and MS of **2a** closely resembled those of **1**. On treatment with 3,5-dinitrobenzoyl chloride, **2a** gave a bis-3,5-dinitrobenzoate (**2c**), which confirmed that the two oxygen atoms were present as hydroxy groups. Thus **2a** possessed an acyclic tetraprenyl structure with two hydroxymethyl groups, one of which was placed on C-1, in view of its splitting pattern (doublet) in the ^{1}H NMR spectrum. **2a** was isomeric to **1** regarding the location of the extra hydroxyl group.

In order to determine the location of the hydroxyl group and the geometries of the double bonds in **2a**, detailed decoupling experiments were attempted by use of Eu(DPM)$_3$ as a shift reagent. By comparison with the ^{1}H NMR spectrum of **1**, all of the protons of **2a** were assigned as listed in Table 1. On irradiation of the signal at δ 5.21 (C-14) in the presence of the shift reagent, both broad singlets at 1.65 and 1.69 (C-16 and C-20) were sharpened showing that these two methyl groups and the olefinic proton were arranged on the same double bond and mutually underwent an allylic long-range coupling. Therefore, the hydroxyl group was situated neither on C-16 nor C-20. On the other hand, as irradiation of the signal at 6.44 (C-2) resulted in sharpening of the signal at 2.02 (C-17) and the collapsing of the doublet at 5.48 (C-1) to a singlet, the hydroxyl group could not be placed on C-17. Consequently, it must be located on C-19.

The geometry of the Δ^2-double bond was assigned as *E* by the $\Delta\delta$ values $\{\Delta\delta = \delta[\text{Eu(DPM)}_3] - \delta(CCl_4)\}$ (see Table 1) for the methyl (C-17) over the methylene groups (C-4) [1] and by the chemical shift of the former [15]. The chemical shift of the olefinic proton (C-10) and the shift