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ISOFLAVONE, WAX AND TRITERPENE CONSTITUENTS OF *WYETHIA MOLLIS*

THOMAS G. WADDELL, MARK H. THOMASSON, MICHAEL W. MOORE, HOUSTON W. WHITE, DOROTHY SWANSON-BEAN, MARGARET E. GREEN, GENE S. VAN HORN* and HENRY M. FALES†

Department of Chemistry and *Department of Biology, University of Tennessee, Chattanooga, TN 37402, U.S.A.; †National Heart and Lung Institute, National Institutes of Health, Bethesda, MD 20014, U.S.A.

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Key Word Index—*Wyethia mollis*; Compositae; *n*-alkanes; wax esters; isoflavones; santal; 3′-*O*-methylorobol; new triterpene; 22, 25-epoxylanota-7:9(11)-dien-3-one.

Abstract—The hexane extract of *Wyethia mollis* contains the *n*-alkanes C_{15}–C_{18}, C_{20}–C_{25}, C_{27} and C_{29}. Linoleic acid was the only detectable acidic component. A mass spectral analysis of the wax ester fraction indicated that it was a mixture of homologues, the saturated even-carbon acids *n*-C_{16}–C_{30} esterfield with the saturated even-carbon alcohols *n*-C_{18}–C_{26}. The chloroform extract yielded the known isoflavones santal and 3′-*O*-methylorobol along with a new lanostane-type triterpene, 22, 25-epoxy-lanosta-7:9(11)-dien-3-one. The wide distribution of *n*-alkanes and the decreased odd–even carbon ratio are consistent with the proposed primitive nature of this plant.

INTRODUCTION

The plant *Wyethia mollis* Gray (Compositae; tribe Heliantheae) is of special interest for two reasons. Firstly, it is both a poisonous plant and a plant with reported folk medicinal value; in particular, the Klamath Indians used *W. mollis* in the treatment of pains, bruises, fevers, and colds[1]. Secondly, it is a plant of taxonomic interest in that it may be the closest living species to an archetype for the Compositae[2, 3]. This view has been challenged by Stuessy[4], who has suggested that *Wyethia* is a relatively advanced member of the tribe Heliantheae. In view of this background, we studied the constituents of *Wyethia mollis* with the hope of clarifying the taxonomic question and determining the chemical basis for the plant's biological activity.

RESULTS AND DISCUSSION

The hexane extract from *W. mollis* was partitioned between aqueous sodium hydroxide and chloroform to give the organic solution containing the alkanes and wax esters and the aqueous solution containing acid constituents. The alkanes and esters were separated and purified by Si gel chromatography. Mass spectral analysis of the wax ester mixture [5] showed it to be a mixture of homologues, the saturated even-carbon acids *n*-C_{16}–C_{30} esterified with the saturated even-carbon alcohols *n*-C_{18}–C_{26}. The aqueous fraction was subjected to GC analysis, with linoleic acid, determined as the methyl ester, as the only detectable acid component.

The *n*-alkane fraction was analysed by GC and the following constituents were identified by comparison with standard reference materials: C_{15}(4.8%), C_{16}(5.6%), C_{17}(11.6%), C_{18}(8.2%), C_{20}(12.6%), C_{21}(9.9%), C_{22}(0.9%), C_{23}(12.2%), C_{24}(6.2%), C_{25}(14.3%), C_{27}(6.6%) and C_{29}(7.1%). This wide distribution of *n*-alkanes in unusual in that plant sources ordinarily yield *n*-alkanes in the C_{25}–C_{35} range with C_{29} and C_{31} predominant[6]. It has been reported that in higher plants, including the Compositae, the content of odd carbon alkanes is usually greater than that of even-numbered *n*-alkanes by a factor of more than ten [6, 7]. The odd–even ratio observed in the *W. mollis* *n*-alkanes is *ca* 2:1. The report of the occurrence of lower carbon number alkanes in more primitive members of a taxon and the reduced predominance of odd over even number carbon alkanes in evolutionarily ancient genera[8] is therefore of significance. Indeed, the leaf wax of the primitive *Ginkgo biloba* L. shows an *n*-alkane range of C_{22}–C_{31} and an odd–even ratio of 1.6:1. Therefore, if the foregoing supposition is correct, our results on the *n*-alkane distribution in *Wyethia mollis* would seem to support a primitive status for this species.

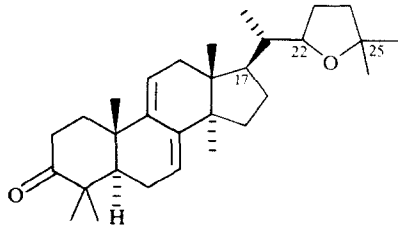
The chloroform extract was subjected to Si gel chromatography and yielded, in order of elution, three crystalline compounds: a new triterpene, identified as 22,25-epoxylanosta-7:9(11)(dien-3-one(1) (see below); the known 3′-*O*-methylorobol(5, 7, 4′-trihydroxy-3′-methoxyisoflavone) [9]; the known santal (5,3′,4′-trihydroxy-7-methoxyisoflavone) [10]. The occurrence of isoflavones in a species of Compositae is surprising. Whereas the distribution of flavonoids in higher plants is widespread, isoflavones are largely confined to the Leguminosae [11]. Although isoflavonoid structures (coumestans and rotenoids) have been reported in Compositae (tribe Heliantheae) [12], Gibbs [13] has no record of isoflavones in Compositae. In reference to the folk medicinal activity of *W. mollis*, it is noted that

isoflavones have been shown to exhibit estrogenic activity [14], to reduce the accumulation of cholesterol in rats [15], to act as central nervous system stimulants [16], and to possess cytotoxic activity [17]. However, the amount of isoflavone present (0.03% dry wt isolated) may not be enough to account for the activity of *W. mollis*.

Compound **1** has a molecular formula of $C_{30}H_{46}O_2$ as determined by high resolution mass spectroscopy ($[M]^+$ at 438.3496, formula requires 438.3497). The UV spectrum of **1** displayed the typical 7:9(11) lanostadiene absorptions (UV λ_{max} nm: 237, 243, 251) [18]. Such a pattern is not consistent with the stereoisomeric 7:9(11) euphadiene absorption[19]. The ^1H NMR spectrum (360 MHz) confirmed the presence of two trisubstituted double bonds (1H vinyl signals at δ5.40 *d* and δ4.52 *q*) and established **1** as a lanostane-type triterpene (quaternary methyl singlets at δ0.88, 1.08, 1.09, 1.14, 1.55, 1.67, 1.72; secondary methyl doublet at δ0.98). The IR spectrum of **1** indicated the nature of one of the two oxygen atoms present in the molecule (sharp ketone absorption at 1709 cm^{-1}). The lack of hydroxyl absorption in the IR along with C–O absorption present at 1114 cm^{-1} was suggestive of an ether linkage. Evidence in support of this feature is seen in the ^1H NMR spectrum where a 1H quartet at δ4.19 ($\rangle$CH–O–)[20] and the two downfield methyl singlets place the ether linkage between C-25 and C-22 (on biogenetic grounds [21]).

The high resolution mass spectrum of **1** is indicative of the proposed structure and supportive of the C-22 to C-25 ether linkage. The base peak is present at *m/z* 423 $[M-Me]^+$ and an $[M-H_2O]^+$ ion is absent, consistent with the lack of hydroxyl functionality [22]. Fragment ions at *m/z* 339.2687 (18% of base peak) and *m/z* 311.2374 (9%) correspond to $C_{24}H_{35}O$ and $C_{22}H_{31}O$ respectively, and indicate cleavage between C-21 and C-22 and between C-17 and C-21 [19]. The molecular ion and other ions seen in the mass spectrum are consistent with **1** [23].

Compound **1** readily forms a tetrahydro derivative ($[M]^+$ at *m/z* 442) and a methoxime ($[M]^+$ at *m/z* 468), providing further proof for its formulation as a ketone diene. The placement of the ketone grouping at C-3 in **1** stands on firm biogenetic grounds [24]. The assignment of the relative stereochemistry at all positions (except C-22) follows from the UV spectrum (see above) and the lanostane structure of **1**. The relative stereochemistry at C-22 remains unknown. Taxonomically, it is noteworthy that although plants of the Compositae are triterpene accumulators, the lanostane carbon skeleton is not characteristic of the family [25].



1

EXPERIMENTAL

Wyethia mollis was collected in June 1972 in Nevada Co., California, and identified by Dr G. S. Van Horn (co-author). A voucher specimen (Van Horn, 422) was deposited in the Herbarium of the University of Tennessee at Chattanooga.

Isolation of n-*alkanes, wax esters, and linoleic acid.* A 799 g sample of the dried and finely ground leaves and stems was exhaustively extracted with hexane. Concn *in vacuo* gave the hexane extract (7.75 g), which was taken up in $CHCl_3$ and extracted with aq. NaOH. Concn of the organic sol. material gave 4.1 g of an oil which yielded purified *n*-alkanes and wax esters upon alumina chromatography, eluting with hexane. Work-up of the aq. NaOH layer in the usual way gave 8 mg linoleic acid, identified as its methyl ester, by GC comparison with an authentic sample. The wax esters were analysed by EIMS (probe) 70 eV in the manner described [5]. The *n*-alkanes were analysed by GC (1 m × 3 mm; FID; 5% SE-30; He flow 20 ml/min). The column temp. was programmed 150°–250° at 10°/min and *n*-alkanes were identified by comparison with standard reference mixtures.

Isolation of 22, 25-*epoxylanosta*-7:9(11)-*dien*-3-*one*(**1**), 3′-O-*methylorobol and santal.* The original plant material (which had been extracted with hexane) was extracted repeatedly with $CHCl_3$. Concn *in vacuo* gave 77 g dark tar which was chromatographed on a column of Si gel eluting with hexane–$CHCl_3$ (1:2) followed by $CHCl_3$ with increasing proportions of Me_2CO. The more polar fractions from the column were combined and re-chromatographed as described to yield, in order of elution, the new triterpene **1** (15 mg), 3′-*O*-methylorobol (101 mg), and santal (136 mg). 3′-*O*-Methylorobol was identified by comparing its mp EIMS, ^1H NMR and UV spectra with published data [9]. Formation of the triacetate derivative, mp 181.5–184.5°, provided confirmation[9]. Santal was identified as described above and by direct comparison (TLC, mmp, IR) with an authentic sample, kindly supplied by Professor W. B. Whalley.

22,25-*Epoxylanosta*-7:9(11)-*dien*-3-*one* (**1**). Mp 168.5–171°; IR ν_{max}^{KBr} cm^{-1}: 1709 (C=O), 1114 (C–O), 870, 835, 805 ($\rangle$C=CH); ^1H NMR (360 MHz, $CDCl_3$): δ 0.98 (3H, *d*) (C-21 Me), 0.88, 1.08, 1.09, 1.14, 1.55, 1.67, 1.72 (21H, *s* each, seven Me groups), 1.80 (1H, *m*), 2.00 (1H, *m*), 2.09 (2H, *s*), 2.40 (1H, *m*), 2.58 (1H, *m*) (allylic protons); 4.19 (1H, *q*) (H-22), 4.52 (1H, *q*) (H-7), 5.40 (1H, *d*) (H-11). EIMS (probe) 70 eV *m/z* (rel. int.): 438.3496 (91) ($C_{30}H_{46}O_2$ requires 438.3497), 423 (100) $[M-Me]^+$, 405 (16), 339.2683 (18) ($C_{24}H_{35}O$ requires 339.2687), 311.2372 (9) ($C_{22}H_{31}O$ requires 311.2374), 271 (18), 257 (14).

Extraction of W. mollis *for sesquiterpene lactones.* A 52.4 g sample of the dried ground plant material was extracted by the standard procedure selective for sesquiterpene lactones [26]. The final extract did not show the typical IR γ-lactone absorption found in these natural products.

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