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DRAMATIC VARIATION IN DITERPENOIDS OF DIFFERENT POPULATIONS OF *BELLARDIA TRIXAGO*

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Key Word Index—*Bellardia trixago* L., Scrophulariaceae, diterpene alcohols, cyclic diterpene biosynthesis

Abstract—A survey of the main components of different populations of *Bellardia trixago* shows a dramatic variation in its diterpenoid constituents. The presence in the same plant of acyclic, monocyclic and bicyclic skeletons reinforces the hypothesis that cyclization of geranylgeraniol to the labdane skeleton follows a pathway with a monocyclic intermediate

INTRODUCTION

In order to continue our research on the chemical reactivity of trixagol (**1**) [1], we were obliged to have at our disposal large amounts of this diterpene alcohol described as the major component in preceding studies on *Bellardia trixago* L. collected in Castilla-León (NW Spain) [2]. For this purpose, we extracted flowers from different populations of this plant, collected in Andalucia (SE Spain), the presence in it of important amounts of diterpene compounds not described in the preceding paper nor in other species of the Scrophulariaceae, made a re-examination of its composition desirable.

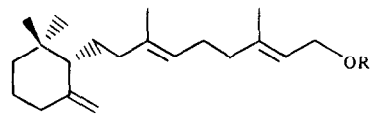
RESULTS AND DISCUSSION

The successive chromatographic separations of the defatted hexane extract of flowers from a mixture of plants collected in different areas of Andalucia, allowed us to identify a total of 16 components, of which trixagol (**1**), 3,4-dihydro-γ-ionona (**2**), α-ambrinol (**3**), dinortrixagona (**4**), isotrixagol (**5**), 5-hydroxyauranetin (**6**), β-sitosterol and ditrixagoyl malonate (**7**) had been already described in *B. trixago* [3]. Identification of such compounds was made by comparison of their physical and spectroscopic properties with those of authentic samples, while the presence of longifolene (**8**) [4], β-cariophyllene (**9**) [5], benzoic acid, calycopterine (**10**) [6], geranylgeraniol (**11**) [7], labda-7,13*E*-dien-15-ol (**12**) [8] and the new natural substances trixagoyl malonate (**13**), labda-7,13*E*-dien-15-yl malonate (**14**) and geranylgeranyl malonate (**15**) was not reported in the preceding paper. The last three substances were isolated by extraction with an aqueous solution of sodium bicarbonate and were characterized as their methyl esters (**16**–**18**). Compound **17** was a colourless oil with IR absorptions at 1747, 1737, 1632 and 1400 cm^{-1}, characteristics of a doubly esterified malonate [9]. Its ^1H NMR spectrum showed signals corresponding to labda-7,13*E*,dien-15-ol with a shift of the C-15 methylene from 4.15 to 4.65 ppm for **17**. This is higher than that observed when it is esterified with monocarboxylic acids and agrees with the shift resulting when similar compounds are esterified with malonic acid [3]. Moreover, two singlets at 3.72 and 3.37 ppm (corresponding to methoxycarbonyl and methylene between two carbonyl groups, respectively) are observed. Saponification of **17** yielded **12**. Compound **18** showed IR and ^1H NMR spectroscopic data that were also in agreement with the proposed structure and, likewise, when saponified gave the corresponding diterpene alcohol **11**. Finally, **16** was identified by comparing its properties with those of an authentic sample [3].

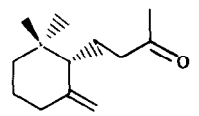
We have found that there exist important differences, both qualitative and quantitative, in the chemical composition of *B. trixago* collected in different areas of the region of Andalucia as compared with that collected in Castilla-León. On the other hand, when samples from different areas were extracted separately, saponified and their diterpene alcohol composition analysed by ^1H NMR spectroscopy, we obtained the results shown in Table 1. It can be observed that their composition changes dramatically depending on the place where they were collected. Thus, in some areas only the acyclic diterpene was found, whereas in other areas one of the cyclics or the acyclic, together with one of the cyclic diterpenes, were found. It is important to note that the

Table 1. Relative composition in major diterpene alcohols in saponified hexane extracts of plants of *Bellardia trixago* L. according to place of collection

Place of collection	Content in **1**	**11**	**12**
La Malaha (Andalucia)	0	25	75
Albuñol (,,)	0	100	0
Alfacar (,,)	66	34	0
Cubillas (,,)	0	100	0
Jaén (,,)	0	0	100
Golpejas (Castilla-León)	100	0	0



1 R = H
13 R = $COCH_2COOH$
16 R = $COCH_2COOMe$



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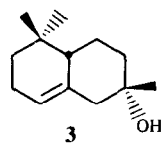

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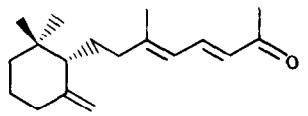

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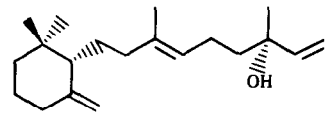

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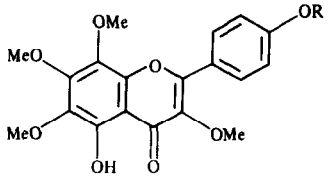
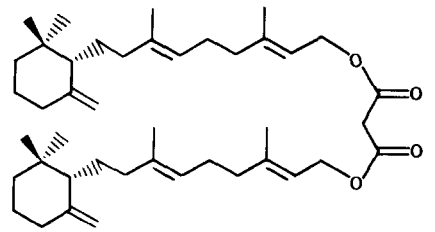


6 R = Me
10 R = H



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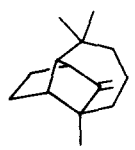

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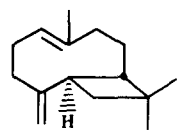

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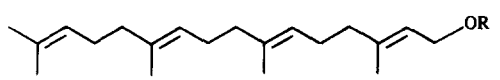
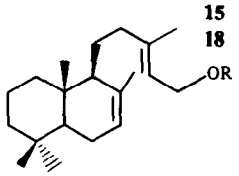
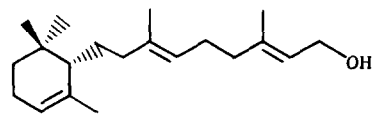


11 R = H
15 R = $COCH_2COOH$
18 R = $COCH_2COOMe$



12 R = H
14 R = $COCH_2COOH$
17 R = $COCH_2COOMe$



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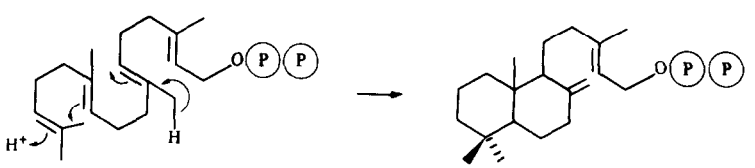

Scheme 1

mono and bicyclic isomers are not found together in the same botanic population

Studies on the biosynthesis of diterpenes have established that geranylgeranyl pyrophosphate is the substrate from which the majority of the diterpene skeletons are derived by a process of cyclization. If it is induced by protonation of the double bond at C-14, it will lead to the direct formation of the bicyclic labdane skeleton as the first step in the synthesis of cyclic diterpene, which later will develop into more complicated skeletons (Scheme 1)

During the last decade [2, 10] two exceptions to this general mechanism have been reported: two substances, **1** and caulerpol (**19**), with a retinane skeleton, have been isolated from different botanic species; these products are closely related to retinol, which is not found in higher plants The isolation of these compounds opens the door to the possibility that the formation of the labdane is preceded by a monocyclic skeleton where the biogenesis may or may not stop In this sense, these results parallel those of biomimetic synthesis. For example, the cyclization of geranylgeranyl derivatives with different chemical reagents normally leads to bicyclic or tricyclic skeletons [11], but under certain conditions [11, 12] the cyclization can be interrupted also forming monocyclic products along with the former ones Moreover, in some primitive animal organisms, such as the marine sponges, monocyclic diterpenes have been found, of which the biosynthetic precursor is the corresponding acyclic derivative [13, 14] The presence of the three diterpene skeletons in *B. trixago* can provide the evidence that, in plants as well, the first step in the biogenetic cyclization of diterpene does not give a labdane ring, but a monocyclic one If, under certain conditions an inhibiting agent of the enzymic system catalysing these reactions was involved, mono or acyclic products would appear

EXPERIMENTAL

The plants were collected during May–June (1985) at Golpejas (Castilla-León, NW Spain), Jaén, La Malaha, Albuñol, Alfacar, Cubillas (Andalucia, SE Spain) and authenticated by Professor F Valle (Departamento de Botanica de la Universidad de Granada) A voucher specimen of each is available for inspection at the herbarium of the Faculty of Sciences of the University of Granada

Hexane extract of a mixture of different plant populations Dried flowers proceding from three different populations of *B trixago* (La Malaha, Jaén and Alfacar) (1 55 kg), were extracted with hexane in a Soxhlet device and defatted as usual (116 g) 18 65 g of this extract were chromatographed on a conventional silica gel column and eluted with mixtures of hexane–Et_2O of increasing polarity Each of the resulting fractions was rechromatographed on a silica–$AgNO_3$ (20% $AgNO_3$) column with the same solvents, the following substances were isolated **8** (53 mg), **9** (11 mg), **2** (120 mg), **3** (30 mg), **7** (130 mg), **4** (60 mg), **6** (161 mg), benzoic acid (60 mg), β-sitosterol (103 mg), **5** (9 mg), **12** (14 mg), **1** (11 mg) and **11** (14 mg) Their spectroscopic properties were compared with reported data The fractions of higher polarity (9 5 g) eluted with Et_2O–MeOH were dissolved in Et_2O and extracted with a $NaHCO_3$ soln On acidification with 2 M HCl and Et_2O extraction, a mixture (1 65 g) of **13**, **14** and **15** was obtained which, after esterification with diazomethane and column chromatography on silica–$AgNO_3$ eluted with hexane–Et_2O mixtures, was separated into its components These substances, when saponified, gave the corresponding alcohols (**1**, **11** and **12**) The Et_2O soln resulting from the extraction with $NaHCO_3$ soln was extracted with a 4% NaOH soln which, on acidification and subsequent extraction with Et_2O, gave a mixture from which **10** (10 mg) was isolated by column chromatography

Methyl labda-7,13E-dien-15-yl malonate (**17**) Viscous liquid IR ν_{max}^{film} cm^{-1} 1747, 1737, 1660, 1400, 1271, 1148, 1021, 981, 798, ^1H NMR (80 MHz, $CDCl_3$) δ0 75 (3H, s, Me-20), 0 85 (3H, *s*, Me-18), 0 87 (3H, *s*, Me-19), 1 70 (6H, s, Me-16 and Me-17) 3 37 (2H, *s*, $-OCOCH_2-COOMe$), 3 72 (3H, s $-OMe$), 4 65 (2H, *d*, $J=7$ Hz, CH_2OCO-), 5 35 (2H, *m*, H-7 and H-14)

Methyl geranylgeranylmalonate (**18**) Viscous liquid, IR ν_{max}^{film} cm^{-1} 1747, 1737, 1663, 1400, 1264, 1149, 1022, 800, ^1H NMR (80 MHz, $CDCl_3$) δ1 60 (9H, s, Me-7, Me-11 and Me-15), 1 70 (6H, s, Me-3 and Me-16), 3 37 (2H, s, $-OCOCH_2COOMe$), 3 75 (3H, s $-OMe$), 4 65 (2H, *d*, $J=7$ Hz, H-1) 5 10 (3H, *m*, H-6), 5 35 (1H, $J=7$ Hz H-2)

Major components of the hexane extract of each of the populations of B trixago *collected in Andalucia* 45 g of plants collected in La Malaha were extracted by refluxing with hexane (1 hr) and after filtration and solvent evapn, the residue (1 8 g) was analysed (^1H NMR monitoring) showing the presence of a mixture of malonates of **1** and **11** The extract was saponified, affording the free alcohols in a 1 3 ratio A similar work-up with samples from different areas was carried out, with the following results Albuñol 50 07 g of sample, 1 68 g of extract, contains **11**, Alfacar 9 80, 0 24, **1** and **11** (2 1), Cubillas 8 97, 0 086, **11**, Jaén 41 90, 1 40, **12**

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