Phytochemistry, Vol. 27, No. 12, pp. 3969–3970, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00
Pergamon Press plc.

GERMACRANOLIDES FROM *SANTOLINA ROSMARINIFOLIA* SUBSP. *CANESCENS*

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(*Received in revised form* 20 *April* 1988)

Key Word Index—*Santolina rosmarinifolia*; Compositae; germacranolides; sesquiterpene lactones.

Abstract—Two sesquiterpene hydrocarbons and six germacranolides have been isolated as the major components of the hexane and ethyl acetate extracts from the flowers of *Santolina rosmarinifolia* subsp. *canescens*. The structures of these substances have been established by means of spectroscopic methods.

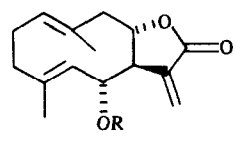
INTRODUCTION

As part of our research programme into the chemical composition of Compositae of Sierra Nevada (Spain), we have studied the hexane and ethyl acetate extracts from the flowers of *Santolina rosmarinifolia* L. subsp. *canescens* which grows at altitudes of 800–1300 m. No previous studies on the chemical components of this plant have been reported, though the composition of *S. rosmarinifolia* subsp. *rosmarinifolia* is partially known; thus, the acetylenic compounds from its roots [1] and the essential oil from its aerial parts have been investigated [2].

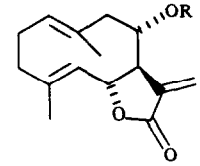
RESULTS AND DISCUSSION

The hexane extract from the flowers of *S. rosmarinifolia* subsp. *canescens* contained the sesquiterpene hydrocarbons germacrene-D [3] and β-cariophyllene as well as five germacranolides. Chamisellin (**1a**) and chamissanthin (**2a**) were identified by ^{1}H NMR [4, 5] and ^{13}C NMR spectroscopy, as well as by acetylation to give the well-known lauranobiolide (**1b**) and tulipinolide (**2b**), respectively. Spiciformin (**3**), 4α,5β-epoxi-6α-hydroxygermacra-1(10),11(13)-dien-8α,12-olide (**4**) and 1α,10β-epoxi-6α-hydroxygermacra-4,11(13)-dien-8α,12-olide (**5**) had spectroscopic properties which were very similar to those reported [6, 7]. The ethyl acetate extract, after acetylation, gave the diacetylderivative of **6** [7].

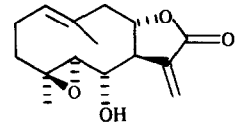
The presence of germacranolides as the major components in *S. rosmarinifolia* subsp. *canescens* shows that there exists an important chemical difference between this and the other species of *Santolina*, from which neither germacranolides nor other sesquiterpene lactones, with the exception of the guayanolides santonine and santoninol (isolated from *S. achillea* [8]), have been isolated.



	R
1a	H
1b	Ac



	R
2a	H
2b	Ac



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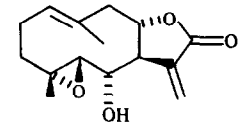

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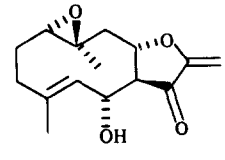

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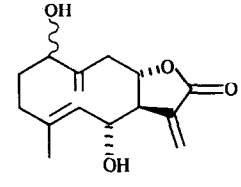

6

EXPERIMENTAL

The plant was collected in June 1985 in Fuente del hervidero (Sierra Nevada, Granada, Spain) and was identified by Professor F. Valle (Departamento de Botánica de la Universidad de Granada). A voucher specimen is available for inspection at the herbarium, Faculty of Sciences, University of Granada. The flowers, were air-dried (500 g) and then extracted with hexane in a Soxhlet. The resulting extract (17.71 g), after defatting with MeOH at −10° (10.12 g), was chromatographed on silica and silica–$AgNO_3$ columns with hexane–Et_2O, giving: germacrene-D (227 mg), β-cariophyllene (21 mg), **1a** (268 mg), **2a** (47 mg), **3** (97 mg), **4** (25 mg) and **5** (59 mg). The 225 g of plant material

remaining from the hexane extraction were treated with EtOAc in a similar way and then chromatographed on silica gel. The fraction eluted with hexane-EtOAc (4:1) (940 mg) was acetylated with Ac_2O–pyridine at room temp. followed by purification on a silica gel column with hexane–Et_2O, affording 257 mg of the diacetyl derivative of **6**.

Chamisellin (**1a**). ^{13}C NMR (20.15 MHz, $CDCl_3$): 126.8 (C-1), 23.0, 24.2 (C-2), 35.9, 38.5 (C-3), 135.8 (C-4), 129.6, 131.9 (C-5), 70.2 (C-6), 50.0, 51.2, 53.9 (C-7), 78.7, 79.8, 83.3 (C-8), 42.3, 47.2 (C-9), 130.4 (C-10), 135.8 (C-11), 170.1 (C-12), 126.8 (C-13), 16.6, 20.7 (C-14), 17.5 (C-15).

Chemissanthin (**2a**). ^{13}C NMR (20.15 MHz, $CDCl_3$); δ129.5 (C-1), 25.8 (C-2), 39.0 (C-3), 127.4 (C-5), 77.1 (C-6), 54.7 (C-7), 71.3 (C-8), 52.8 (C-9), 169.3 (C-12), 126.6 (C-13), 17.2 (C-14), 17.7 (C-15).

4β,5α-Epoxi-6α-hydroxygermacra-1(10),11(13)-dien-8α,12-olide (**3**). ^{13}C NMR (20.15 MHz, $CDCl_3$): δ 127.2 (C-1), 23.4 (C-2), 37.3 (C-3), 61.3 (C-4), 64.7 (C-5), 68.8 (C-6), 46.0 (C-7), 77.1 (C-8), 43.2 (C-9), 129.9 (C-10), 124.6 (C-11), 169.6 (C-12), 127.8 (C-13), 15.9 (C-14), 19.3 (C-15).

Diacetyl derivative of **6**. $[\alpha]_D - 75.1°$ ($CHCl_3$; *c* 1.0); MS (probe) 70 ev, *m/z* (rel. int.): 306 $[M-CH_2=C=O]^+$ (1), 289 $[M-MeCOO]^+$ (5), 288 $[M-AcOH]^+$ (2), 246 $[M-AcOH-CH_2=C=O]^+$ (30), 228 $[M-AcOH-AcOH]^+$ (80), 213 (50); ^1H NMR (80 MHz, $CDCl_3$): δ 4.75–5.05 (3H, *m*, H-1, H-5, H-6), 2.9 (1H, *m*, H-7), 4.03 (1H, *m*, H-8), 5.74 (1H, *dd*, 1, 2.5, H-13), 6.23 (1H, *dd*, 1, 2.5, H-13'), 1.79 (3H, *br s*, H-14), 5.16 (2H, *m*, H-15), 1.98 (3H, OAc), 2.0 (3H, OAc).

Acknowledgements—We thank Professor F. Valle (Departamento de Botánica de la Facultad de Ciencias de la Universidad de Granada) for the identification of the plant material.

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Phytochemistry, Vol. 27, No. 12, pp. 3970–3972, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00
Pergamon Press plc.

[1β,2β,3β-$^3H_3$]GIBBERELLIN A_{20}: CONFIRMATION OF STRUCTURE BY ^3H NMR AND BY MASS SPECTROMETRY

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(*Received* 19 *April* 1988)

Key Word Index—Gibberellin A_{20}; tritiogenation of gibberellin A_5; tritium-labelling; regio-selectivity; ^3H NMR.

Abstract—[1β,2β,3β-$^3H_3$]Gibberellin A_{20}, 55 Ci/mmol, has been prepared by catalytic reduction of gibberellin A_5 methyl ester 13-acetate 16,17-epoxide, followed by deoxygenation of the epoxide and aqueous alkaline hydrolysis. The regio- and stereo-selectivity of labelling has been established by NMR and mass spectrometry.

INTRODUCTION

Gibberellin A_{20} (**1**), stereospecifically labelled with tritium at the 2β- and 3β- positions, was required for studies with enzymes that catalyse the 2β- and 3β-hydroxylation and 2,3-didehydrogenation of gibberellin A_{20} (GA_{20}) (**1**). Murofushi *et al.* [1] have prepared GA_{20} (**1**) by catalytic hydrogenation of GA_5 methyl ester (**3**) but found it difficult to separate GA_{20} methyl ester (**2**) from the complex mixture of reduction products. To avoid this problem, Murofushi *et al.* [2] protected the 16,17-double bond in GA_5 methyl ester by forming the 16,17-epoxide (**5**), then reduced the 2,3-double bond catalytically with a mixture of tritium and hydrogen gas. Deoxygenation of the reduced epoxide by the method of Cornforth *et al.* [3], followed by hydrolysis of the methyl ester with aqueous sodium hydroxide gave tritiated GA_{20}, presumed to be [2,3-$^3H_2$]GA_{20}, but the stereo- and regio- specificity of the tritium atoms was not established. To determine the stereo- and regio-selectivity of catalytic reduction of the