PERGAMON

Phytochemistry 52 (1999) 1055–1058

PHYTOCHEMISTRY

Antifeedant activity of *neo*-clerodane diterpenoids from *Teucrium fruticans* and derivatives of fruticolone

Maurizio Bruno[a], Rosaria Ciriminna[b], Franco Piozzi[a,b,*], Sergio Rosselli[a], Monique S.J. Simmonds[c]

[a]*Dipartimento Chimica Organica, Università di Palermo, Viale d. Scienze, 90128 Palermo, Italy*
[b]*I.C.T.P.N.-C.N.R. (associated with Istituto Nazionale Chimica Sistemi Biologici), La Malfa 153, 90146 Palermo, Italy*
[c]*Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK*

Received 25 March 1999; accepted 9 July 1999

Abstract

The antifeedant activity of three *neo*-clerodane diterpenoids, fruticolone, isofruticolone and fruticolide from *Teucrium fruticans* was assessed using larvae of *Spodoptera littoralis*. Isofruticolone was one of the most potent of the *Teucrium* derived *neo*-clerodanes. Chemical modification of functional groups on fruticolone showed that its activity could be enhanced.

Keywords: *Teucrium fruticans*; Labiatae; *Neo*-clerodane; Diterpenoids; Chemical transformations; *Spodoptera littoralis*; Antifeedant

1. Introduction

Several natural furo-clerodanes are known for their antifeedant activity against insects, and the genus *Teucrium* (family Labiatae) is one of the richest source of such products (Piozzi, 1998). Despite the interest in these compounds it is still not possible to predict the potency of a clerodane compound as the importance of the functional groups remain unresolved (Simmonds & Blaney, 1992). In this study we have evaluated the activity of three *neo*-clerodanes, including fruticolone, the most abundant clerodane in *Teucrium fruticans* L., a species widespread in Sicily (Savona et al., 1978) and known to have antifeedant activity against *Spodoptera littoralis* (Simmonds & Blaney, 1992). We also report the preparation and antifeedant activity of synthetic derivatives, made to further our understanding of the importance of the substitutions on the decaline portion of the furo-clerodane molecule.

2. Results and discussion

Fruticolone was isolated from *T. fruticans* as described previously (Savona et al., 1978). The $NaBH_4$ treatment of fruticolone (**1**) yielded the diol (**2**) in which the 6-keto group was reduced and the new hydroxy group assumed the 6αOH equatorial configuration. As a by-product, the triol (**3**) was obtained, arising from the spontaneous deacetylation of the 5α-CH_2OAc group. Products (**2**) and (**3**) were obtained in 60% and 20% yield, respectively.

By Ac_2O-py treatment (3 h) both products (**2**) and (**3**) gave the diacetyl derivative (**4**). The triacetyl derivative (**5**) was obtained only by longer treatment (48 h) of products (**2**), (**3**) and (**4**), due to the more difficult acetylation of the hindered axial 1α-OH.

Fruticolone (**1**) itself yielded, by prolonged (48 h) Ac_2O-py treatment, the diacetyl derivative (**6**), that by $NaBH_4$ reduction and spontaneous deacetylation of the 5α-CH_2OAc group gave the monoacetate diol (**7**) with 6α-OH equatorial configuration. Product (**7**) was also transformed into the triacetyl derivative (**5**).

The acetylation was performed also on isofrutico-

* Corresponding author. Tel.: +39-91-596905; fax: +39-91-596825.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00385-4

Table 1
Effect of compounds on the feeding behaviour of *Spodoptera littoralis*

Feeding index[a]		
Compounds[b]	Mean	(s.e.m.)
(**1**) Fruticolone	29	(7.4)
(**2**) Diol	49	(16.1)
(**3**) Triol	16	(13.8)
(**4**) Diacetate	60[c]	(9.8)
(**5**) Triacetate	14	(11.7)
(**6**) Diacetyl-fruticolone	−5	(5.2)
(**7**) Diol	27	(8.5)
(**8**) Isofruticolone	53[c]	(12.6)
(**9**) Diacetyl-isofruticolone	−27	(9.3)
(**10**) Orthoacetate	12	(5.6)
(**11**) Diketone	53[c]	(5.3)
(**12**) Fruticolide	20	(15.5)

[a] Feeding index = $((C-T)/(C+T))\%$, where C is amount of control discs, and T the amount of treatment discs eaten after an 18 h bioassay; +ve Index indicates an antifeedant and a −ve Index indicates a phagostimulant.
[b] Compounds applied to discs at 100 ppm.
[c] Significant $P < 0.05$, Wilcoxon matched pairs test, $n = 20$.

lone (**8**), a minor constituent of *T. fruticans* (Savona et al., 1978), obtaining the diacetyl derivative (**9**) in which the secondary hydroxy group retained the original 6β-OH axial configuration.

A remarkable reaction of fruticolone arises from its heating at 200°C under nitrogen atmosphere: an orthoacetate triester (**10**) is formed, involving three hydroxy groups (4α, 6α and 19): the epoxide ring is opened, and an 18β–6β ethereal bridge is formed. This reaction had been previously observed on other analogous *neo*-clerodane derivatives (de la Torre, Fernandez & Rodriguez, 1987).

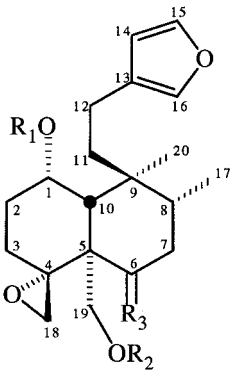
We tested the antifeedant activity of compounds (**1**–**10**) and: (a) the previously described diketone (**11**) obtained by chromic oxidation of both fruticolone (**1**) and isofruticolone (**8**) (Savona et al., 1978); (b) fruticolide (**12**), a minor extractive of *T. fruticans* (Bruno et al., 1992).

The relative and absolute configuration of fruticolone (**1**) and isofruticolone (**8**) was proved by CD curve determination and by X-ray diffraction experiments (Savona et al., 1978; Martinez-Ripoll et al., 1981).

3. Biological activity

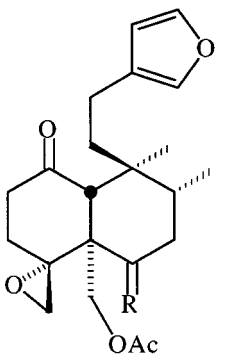
The results are reported in Table 1. Fruticolone (**1**) showed some antifeedant activity against *Spodoptera littoralis*; this activity was lost when transformed into the diacetyl derivative (**6**) but increased in the 1,6-diketone (**11**). With fruticolone, the reduction of the keto group at C-6 to a 6α-OH function increases the antifeedant activity, as shown by 1,6-diol (**2**) and the 6,19-diacetyl derivative (**4**). In contrast, there is a loss of activity in the 1,6,19-triol (**3**), its triacetyl derivative (**5**) and the 1-monoacetyl derivative (**7**). These results can be rationalised as follows: the occurrence of a 1-ketone or free 1α-OH group, of a acetylated 5α-CH_2OAc group and/or of a 6-ketone, 6α-OH, or 6α-OAc function are associated with antifeedant activity, whereas there is a decrease in activity from the acetylation of the 1-OH and from the deacetylation of 5α-CH_2OAc.

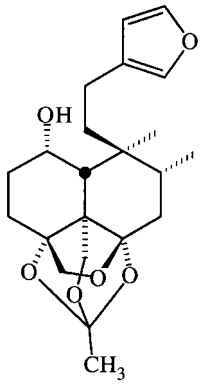
Isofruticolone (**8**) and the diketone (**11**) have potent antifeedant activity, but this activity is lost in the 6,19-diacetylderivative (**9**). suggests that the presence of a 1-ketone is not critical for activity, but the orientation and composition of the substitute on C-6 are important. Both (**8**) and (**9**) have either axial 6β-OH or 6β-OAc functions, respectively, instead of the equatorial 6α-OH or 6α-OAc occurring in the fruticolone derivatives (**2**) and (**4**), respectively. Whether this difference or the composition of the substitute at C-1 explains the difference in activity between compounds (**4**) and (**9**) justifies further research. Neither the orthoacetate (**10**) or fruticolide (**12**) had potent antifeedant activity.



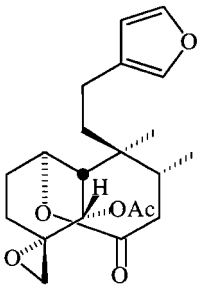
	R_1	R_2	R_3
1	H	Ac	O
2	H	Ac	α-OH, β-H
3	H	H	α-OH, β-H
4	H	Ac	α-OAc, β-H
5	Ac	Ac	α-OAc, β-H
6	Ac	Ac	O
7	Ac	H	α-OH, β-H

	R
8	β-OH, α-H
9	β-OAc, α-H
11	O





10



12

4. Experimental

4.1. General experimental procedures

IR spectra (nujol) were registered on a Perkin-Elmer 1310 spectrophotometer. ^{1}H-NMR spectra were determined on a Bruker 250 instrument. Mass spectra were registered on a VG ZAB 2F instrument (EI 70 eV, source temperature 180°C) by the MS C.N.R. Service at Padova. Column chromatographies on silicagel Merck no. 7734, deactivated with 15% H_2O, and radial chromatography with a Harrison Chromatotron 8924 instrument were used for the purification of the products.

4.2. Reduction of fruticolone (*1*) to 1,6-diol (*2*) and 1,6,19-triol (*3*)

Fruticolone (**1**) (500 mg) dissolved in MeOH (50 ml) was treated with $NaBH_4$ (500 mg) at room temperature for 10 min. Usual treatment and chromatographic separation yielded the 1,6-diol (**2**) (300 mg) and the 1,6,19-triol (**3**) (100 mg). Diol (**2**), vitreous solid: IR ν_{max} 3350, 1735, 1250, 885 cm^{-1}. MS *m/e* 390 (M^+), 374, 332, 314, 284, 220, 202, 95, 81 (100%). ^{1}H-NMR. Triol (3), amorphous solid: IR ν_{max} 3280, 870 cm^{-1}. MS *m/e* 332 (M-H_2O), 314, 284, 190, 95, 81 (100%). ^{1}H-NMR spectral data are shown in Table 2.

Table 2
^{1}H-NMR spectral data (250 MHz, in $CDCl_3$, TMS as internal standard)

	2	**3**	**4**	**5**
H-1	4.35 m	4.17 m	4.36 m	5.45 m
H-6	3.53 dd	3.57 dd	4.67 dd	4.67 m
	J 3 Hz, J 10 Hz	J 3 Hz, J 10 Hz	J 3 Hz, J 10 Hz	
H-14	6.26 m	6.27 m	6.26 m	6.28 m
H-15	7.35 m	7.35 m	7.36 m	7.32 m
H-16	7.21 m	7.22 m	7.22 m	7.20 m
H-17	0.88 d	0.86 d	0.85 d	0.80 d
	J 6 Hz	J 6 Hz	J 6 Hz	J 6 Hz
H_A-18	2.45 d	2.58 d	2.24 d	2.40 d
	J 4.5 Hz	J 4.5 Hz	J 4.5 Hz	J 4.5 Hz
H_B-18	3.27 d	3.40 d	3.07 d	3.03 d
	J 4.5 Hz	J 4.5 Hz	J 4.5 Hz	J 4.5 Hz
H_A-19	4.26 d	4.20 d	4.91 d	4.96 d
	J 13.5 Hz	J 13.5 Hz	J 13.5 Hz	J 13.5 Hz
H_B-19	5.48 d	4.32 d	5.17 d	5.05 d
	J 13.5 Hz	J 13.5 Hz	J 13.5 Hz	J 13.5 Hz
H-20	1.09 s	1.14 s	1.12 s	0.86 s
OAc	2.10 s	–	1.97 s	1.94 s
OAc	–	–	2.12 s	2.03 s
OAc	–	–	–	2.09 s
	6	**7**	**9**	**10**
H-1	5.51 m	5.40 m	–	4.28 m
H-6	–	3.61 m	4.70 dd	–
			J 3 Hz, J 10 Hz	
H-10	n.o.	n.o.	3.18 s	n.o.
H-14	6.30 m	6.27 m	6.25 m	6.25 m
H-15	7.37 m	7.32 m	7.21 m	7.36 m
H-16	7.23 m	7.21 m	6.35 m	7.20 m
H-17	0.91 d	0.82 d	0.81 d	0.87 d
	J 6 Hz	J 6 Hz	J 6 Hz	J 6 Hz
H_A-18	2.38 d	3.20 d	2.80 d	4.16 s
	J 4.5 Hz	J 4.5 Hz	J 4.5 Hz	
H_B-18	3.45 d	2.40 d	2.65 d	3.90 s
	J 4.5 Hz	J 4.5 Hz	J 4.5 Hz	
H_A-19	4.97 d	4.45 d	4.10 d	4.37 s
	J 13.5 Hz	J 13.5 Hz	J 13.5 Hz	
H_B-19	5.22 d	4.66 d	3.68 d	4.78 s
	J 13.5 Hz	J 13.5 Hz	J 13.5 Hz	
H-20	1.10 s	0.82 s	1.07 s	1.07 s
OAc	2.07 s	2.10 s	1.99 s	–
OAc	2.13 s	–	2.17 s	–
OAc	–	–	–	–
O_3CMe	–	–	–	1.49 s

4.3. Diacetylderivative (*4*) and triacetylderivative (*5*)

The 1,6-diol (**2**) (200 mg) dissolved in anhydrous pyridine (10 ml) was treated with Ac_2O (2 ml) at room temperature for 3 h. Usual work-up gave (**4**) (220 mg), thick oil, IR ν_{max} 3400, 1750, 1270, 875 cm^{-1}. MS: *m/e* 434 (M^+), 416, 284, 202, 149, 95, 81 (100%). The

same treatment for 48 h yielded (**5**) (230 mg), thick oil, IR ν_{max} 1740, 1250, 875 cm^{-1}. MS: *m/e* 476 (M^+), 302, 202, 190, 171, 95, 81 (100%). ^{1}H-NMR spectral data are shown in Table 2.

The treatment of the 1,6,19-triol (**3**) (50 mg) in the same way gave (**4**) (55 mg) after 3 h or (**5**) (60 mg) after 48 h. The treatment of (**4**) in the same way for 48 h yielded (**5**).

4.4. Acetylation of fruticolone (*1*) to diacetylderivative (*6*)

Fruticolone (400 mg) dissolved in anhydrous pyridine (15 ml) was treated with Ac_2O (3 ml) at room temperature for 48 h. Usual work-up gave (**6**) (350 mg), thick oil: IR ν_{max} 1725, 1250, 870 cm^{-1}. MS: m/e 432 (M^+), 360, 300, 206, 95, 81 (100%). ^{1}H-NMR spectral data are shown in Table 2.

4.5. Reduction of diacetylderivative (*6*) to the diol (*7*)

The diacetylderivative (**6**) (200 mg) dissolved in MeOH (20 ml) was treated with $NaBH_4$: usual work-up gave the monoacetate diol (**7**) (150 mg), amorphous solid: IR ν_{max} 3300, 1730, 1250, 865 cm^{-1}. MS: *m/e* 392 (M^+), 374, 285, 190, 95, 81 (100%). ^{1}H-NMR spectral data are shown in Table 2. Product (**7**) was transformed into the triacetylderivative (**5**) by treatment with Ac_2O in pyridine for 48 h.

4.6. Acetylation of isofruticolone (*8*): diacetylderivative (*9*)

Isofruticolone (150 mg) was dissolved in pyridine (8 ml) and treated with Ac_2O (2 ml) for 48 h: the diacetylderivative (**9**) (100 mg), thick oil, was isolated. IR ν_{max} 1725, 1260, 875 cm^{-1}. MS: *m/e* 432 (M^+), 282, 187, 95, 81 (100%). ^{1}H-NMR spectral data are shown in Table 2.

4.7. Pyrolysis of fruticolone: orthoacetate (*10*)

Fruticolone (**2**) (250 mg) was heated at 200°C under nitrogen atmosphere for one hour. After usual work-up the orthoacetate (**10**) (150 mg), vitreous solid, was isolated. IR ν_{max} 3320, 875 cm^{-1}. MS: *m/e* 390 (M^+), 372, 318, 218, 149, 95, 81 (100%). ^{1}H-NMR spectral data are shown in Table 2.

4.8. Antifeedant bioassay

A binary choice feeding bioassay using glass-fibre discs was used to evaluate the activity of the compounds against final stadium larvae of *Spodoptera littoralis* (Simmonds, Blaney & Schoonhoven, 1992). The results are reported in Table 1.

Acknowledgements

We thank Paul Green for help with the rearing of *Spodoptera littoralis* which were cultured under a MAFF licence issues under the Import and Export (Plant Health Great Britain) Order 1980 and the Plant Pests (Great Britain) Order 1980. The present paper was supported by C.N.R.-I.C.T.P.N., MURST 40% and MURST 60% research funds.

References

Piozzi, F. (1998). *Heterocycles*, *48*, 2185.

Simmonds, M. S. J., & Blaney, W. M (1992). In R. M. Harley, & T. Reynolds, *Advances in Labiatae science* (pp. 375–392). Kew, UK: Royal Botanic Gardens.

Savona, G., Passannanti, S., Paternostro, M. P., Piozzi, F., Hanson, J. R., Hitchcock, P. P., & Siverns M. (1978). J. Chem. Soc. Perkin Trans. I, 356.

de la Torre, M. C., Fernandez, P., & Rodriguez, B. (1987). *Tetrahedron*, *43*, 4679.

Bruno, M., Alcazar, R., de la Torre, M. C., Piozzi, F., Rodriguez, B., Savona, G., Perales, A., & Arnold, N. A. (1992). *Phytochemistry*, *31*, 3531.

Martinez-Ripoll, M., Fayos, J., Rodriguez, B., Garcia-Alvarez, M. C., Savona, G., Piozzi, F., Paternostro, M. P., & Hanson, J. R. (1981). J. Chem. Soc. Perkin Trans. I, 1186.

Simmonds, M. S. J., Blaney, W. M., & Schoonhoven, L. M. (1992). *J. Insect Physiology*, *38*, 249.