PERGAMON

Phytochemistry 58 (2001) 463–474

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Semisynthetic derivatives of *ent*-kauranes and their antifeedant activity

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Received 28 November 2000; received in revised form 9 May 2001

Abstract

Chemical modification of functional groups on linearol yielded several *ent*-kaurane derivatives. These compounds were tested for their antifeedant activity against larvae of *Spodoptera littoralis*. Although linearol did not influence the feeding behaviour of larvae, some of its derivatives showed significant antifeedant activity.

Keywords: Lamiaceae; *ent*-Kaurane; Diterpenoids; Linearol; Chemical modifications; *Spodoptera littoralis*; Antifeedant

1. Introduction

ent-Kauranes are naturally occurring diterpenoids isolated from several families, such as Asteraceae and Lamiaceae. These compounds have attracted interest because of their structures and their biological activities as anti-tumorals, anti-HIV and anti-bacterials (Hanson, 1999).

The genus *Sideritis* (family Lamiaceae) is a rich source (Gonzales et al., 1990) of *ent*-kauranes. In a previous paper we described the isolation and identification (Bondì et al., 2000) of many *ent*-kauranes from several Turkish species of *Sideritis*. In the same paper, the antifeedant activity against larvae of *Spodoptera frugiperda* and *S. littoralis* was described. One of the diterpenes, sideroxol (**1**), isolated from *Sideritis akmanii* and *S. rubriflora* had potent antifeedant activity against *S. frugiperda*.

We were able to isolate large amounts of another *ent*-kaurane, linearol (**2**), from species of *Sideritis* (Bondì et al., 2000). This provided us with enough starting material to plan a structure–activity study in which we would modify the different functional groups on the *ent*-kaurane skeleton to evaluate their importance in the antifeedant activity of these compounds.

2. Results and discussion

Linearol (**2**), possessing an exocyclic double bond between carbons 16 and 17, was subjected to catalytic hydrogenation on 10% Pd/C. The reduction that diastereoselectively happened from the sterically less congested side (Piozzi et al., 1971), yielded a mixture of two compounds. After separation, the less polar compound was identified as 16-α-H dihydrolinearol (**3**); the second one (**4**), surprisingly was the transacetylated product of the former, in which the acetyl group migrated from position 18 to position 3.

Since the antifeedant sideroxol (**1**) had an epoxy group we performed an epoxidation on the double bond of linearol. Treatment with 3-chloroperbenzoic acid (MCPBA) in CH_2Cl_2 allowed us to generate, diastereoselectively, the epoxy ring (Piozzi et al., 1973; Gonzales et al., 1990). Also in this case, as described before, we obtained two compounds: epoxylinearol (**5**) and the 18→3 transacetylated product (**6**). Both **5** and **6** were subjected to acetylation to give the same triacetylderivative (**7**).

Next, in order to remove the exocyclic double bond we performed an ozonolysis. Treatment of **2** with ozone

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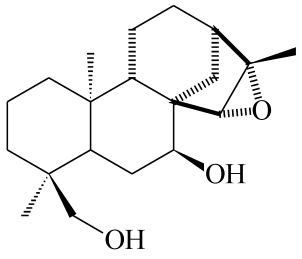
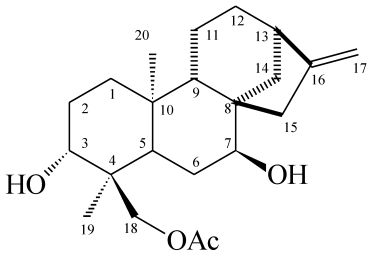
0031-9422/01/$ - see front matter
PII: S0031-9422(01)00252-7



1



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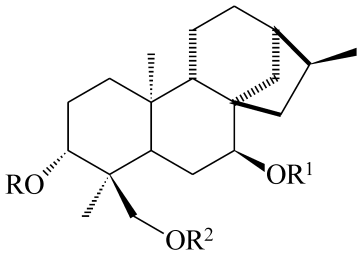
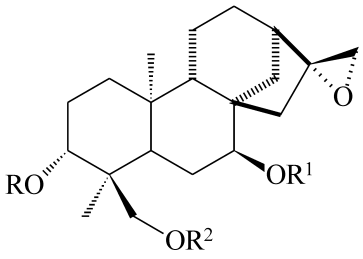
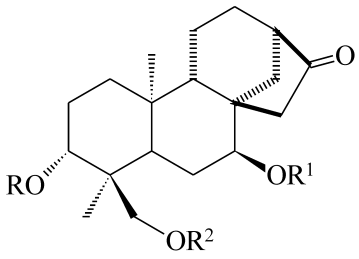


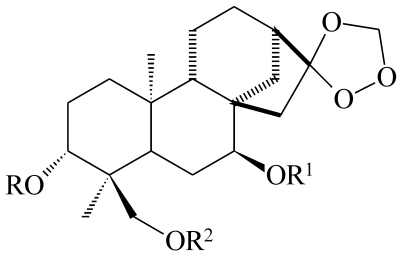
	R	R^1	R^2
3	H	H	Ac
4	Ac	H	H



	R	R^1	R^2
5	H	H	Ac
6	Ac	H	H
7	Ac	Ac	Ac



8 R = R^1 = H R^2 = Ac

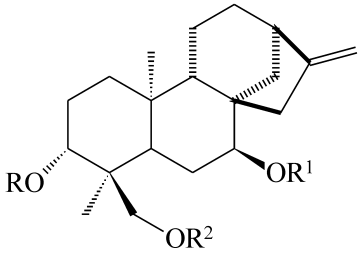


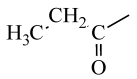
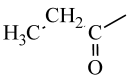
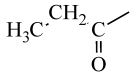
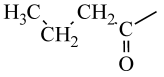
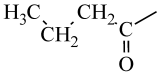
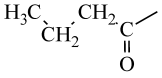
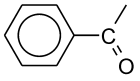
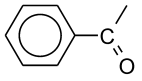
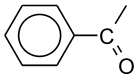
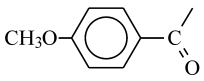
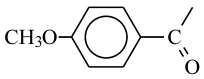
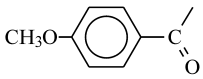
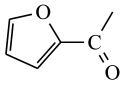
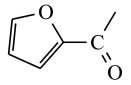
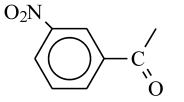
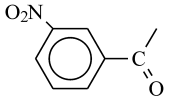
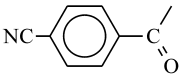
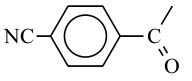
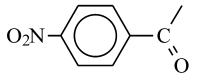
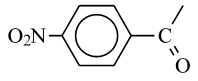
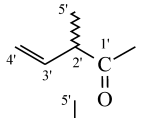
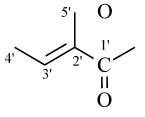
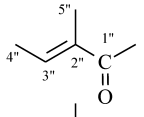
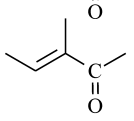
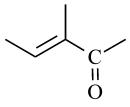
9 R = R^1 = H R^2 = Ac

in CH_2Cl_2 resulted in a mixture of two products. One of them was the expected ketone (**8**), the other one showed in ^{1}H NMR spectrum a signal at δ 5.01 (*br s*, 2H) for acetalic protons and in ^{13}C NMR spectrum two signals at ppm 116.0 (*s*) and 93.2 (*t*) for two carbons bearing two oxygens. Therefore this compound was assigned the structure of ozonide (**9**). Furthermore, **9** was transformed to ketone (**8**) by reduction with Zn/AcOH. Isolation of ozonides is not common, due to their instability; in fact, we could not store **9** as after a few hours it started to decompose.

The composition of ester moieties on neoclerodane diterpenoids influences their antifeedant activity (Rodríguez et al., 1999). Therefore, to evaluate the effect of different esters on the activity of *ent*-kauranes, esterification reactions were undertaken on the two free hydroxyl groups of linearol.

Treatment of linearol with propanoyl chloride, triethylamine (TEA), 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 gave 3,7-dipropanoate ester (**10**) and a mono esterified derivative. The ^{1}H and ^{13}C NMR spectra of the latter compound showed signals at δ 3.60 (*t*, J = 3.8 Hz, H-7) and at δ 4.86 (*dd*, J = 11.2 and 5.2 Hz, H-3) and ppm 76.7 (*d*, C-7) and 74.2 (*d*, C-3), clearly indicating that only 3-OH was esterified; therefore, we assigned to it structure (**11**). Similarly, we prepared



	R	R^1	R^2
10			Ac
11		H	Ac
12		H	Ac
13			Ac
14		H	Ac
15			Ac
16		H	Ac
17			Ac
18			Ac
19			Ac
20			Ac
21			Ac
22		H	Ac
25			Ac
26	Ac		

3-mono- and 3,7-diester derivatives (**12**), (**13**), (**14**), (**15**), (**16**) and (**17**) using different acyl chlorides such as butanoyl, benzoyl and 4-methoxybenzoyl chlorides. The same reactions performed with 2-furoyl chloride, 3-nitrobenzoyl chloride, 4-cyanobenzoyl chloride and 4-nitrobenzoyl chloride allowed us to isolate compounds (**18**), (**19**), (**20**) and (**21**) in which both hydroxyl groups were esterified. Monoacyl derivatives were not obtained.

Acylation of linearol with tigloyl chloride was not an easy transformation. In fact, treatment of **2** with tiglic acid chloride, TEA, DMAP in CH_2Cl_2 gave an inseparable mixture of two compounds. The ^1H and ^{13}C NMR spectra showed that esterification was achieved only in position 3 (δ_H 4.86 *dd*, H-3; δ_C 74.3 *d*/74.4 *d* C-3) and that the ester moiety was the $\Delta_{3'\text{-}4'}$ isomer of tiglate (δ_C 137.2 *d*/137.1 *d*, C-3′; 115.9 *t*/115.7 *t*, C-4′). There-

fore, compound (**22**) was an epimeric mixture at C-2′ due to the formation of a stereogenic center, a consequence of isomerization. Isomerization of a tigloyl moiety has been already observed by other authors (Hatakeyama et al., 1985).

A different behaviour was observed when linearol and tigloyl chloride were refluxed in toluene for 30 min. In this case an inseparable mixture of di-tiglates (**23**) and (**24**) was obtained, in which the double bond between carbons 16 and 17 was hydrated to give a tertiary hydroxyl group in C-16 (δ_H 1.35, CH_3-17; δ_C 78.6 *s*/78.7 *s*, C-16, 24.3 *q*, C-17). Compound (**23**) was an isomer of **24** in which the acetyl group migrated to position 3 and the tiglic ester was at position 18. Finally, by refluxing linearol, TEA and tigloyl chloride in toluene for 24 h two compounds were isolated. The more polar one was identified as 3,7-ditigloyl-linearol (**25**); the other one (**26**) was the transesterification isomer of the former with the tigloyl moiety in position 18 and the acetyl in position 3.

3. Biological activity

The effects of compounds (**2**–**8**, **10**, **11**, **13**–**22**, **25**–**26**) on the feeding behaviour of larvae of *S. littoralis* are reported in Table 2. Linearol did not influence the feeding behaviour of *S. littoralis*. However, modification to the substitutions on the molecule resulted in a range of analogue esters that differ in their activity when tested at 100 ppm. We selected this dose to allow us to better discriminate among the effects of different substances although, in our experience, many diterpenes show activity at 1000 ppm.

Compounds with a ketone or epoxide group had significant antifeedant activity except for **7**. Modification to the substitutions at R (C-3), R^1 (C-7) or R^2 (C-18) altered the activity of the compounds but there were no clear trends in structure–activity relationship. For example, increasing the length of the substitution at R (**11** vs. **22**), when the substitutes at R^1 and R^2 did not change, resulted in an increase in antifeedant activity. However, the activity of compounds varied when similar substitutes were added to both R and R^1, while R^2 was an acetyl group. Of the compounds tested, **26** was the most active; this was the only compound in which the substitution at R^2 was neither an hydroxy nor an acetyl group. This suggests that the composition and position of the functional groups at C-3, C-7 and C-18 modulate the activity of the compound. This is supported by previous research into the antifeedant activity of *neo*-clerodanes from *Teucrium* (Simmonds et al., 1989; Simmonds and Blaney, 1992), *Scutellaria* (Cole et al., 1990) or *Salvia* (Simmonds et al., 1996). Whilst there appears to be no clear structure–activity relationship the

Table 1a
^{13}C NMR spectral data of compounds[a]

C	2	3	4	5	6	7	8	9
1	38.2, *t*	38.4, *t*	38.4, *t*	38.3, *t** [b]	38.0, *t**	37.5, *t**	37.9, *t*	37.8, *t*
2	26.0, *t*	26.4, *t*	23.4, *t*	26.4, *t*	23.3, *t*	22.7, *t*	26.2, *t**	26.1, *t**
3	71.5, *d*	72.3, *d*	74.5, *d*	72.1, *d*	74.4, *d*	73.5, *d*	71.7, *d*	71.8, *d*
4	41.2, *s*	41.8, *s*	41.6, *s*	41.8, *s*	41.6, *s*	40.1, *s*	41.5, *s*	41.4, *s*
5	37.9, *d*	38.2, *d*	37.3, *d*	38.1, *d*	37.2, *d*	39.4, *d*	37.7, *d*	37.8, *d*
6	26.9, *t*	27.2, *t*	26.5, *t*	27.4, *t*	26.6, *t*	23.9, *t*	26.4, *t**	26.7, *t**
7	76.3, *d*	77.1, *d*	77.0, *d*	76.6, *d*	76.5, *d*	78.9, *d*	75.8, *d*	76.1, *d*
8	47.6, *s*	48.5, *s*	48.5, *s*	49.0, *s*	49.1, *s*	47.5, *s*	46.2, *s*	46.6, *s*
9	49.9, *d*	51.4, *d*	51.5, *d*	50.1, *d*	49.7, *d*	49.9, *d*	49.0, *d*	50.0, *d*
10	38.4, *s*	38.7, *s*	38.4, *s*	38.8, *s*	38.5, *s*	38.4, *s*	38.7, *s*	38.5, *s*
11	17.5, *t*	18.3, *t*	18.3, *t*	18.9, *t*	18.9, *t*	18.6, *t*	17.9, *t*	17.3, *t*
12	33.2, *t*	26.3, *t*	26.3, *t*	29.3, *t*	29.4, *t*	28.9, *t*	29.6, *t*	25.6, *t*
13	43.4, *d*	39.5, *d*	39.5, *d*	42.4, *d*	42.5, *d*	42.1, *d*	47.3, *d*	43.3, *d*
14	38.0, *t*	39.8, *t*	39.8, *t*	37.2, *t**	37.1, *t**	36.8, *t**	35.5, *t*	35.9, *t*
15	44.7, *t*	44.4, *t*	44.3, *t*	45.0, *t*	44.9, *t*	44.7, *t*	51.3, *t*	46.5, *t*
16	154.9, *s*	34.4, *d*	34.4, *d*	66.2, *s*	66.3, *s*	65.5, *s*	222.2, *s*	116.0, *s*
17	103.0, *t*	15.6, *q*	15.6, *q*	50.3, *t*	50.3, *t*	50.5, *t*		93.2, *t*
18	65.8, *t*	66.1, *t*	64.2, *t*	65.9, *t*	63.8, *t*	64.6, *t*	65.7, *t*	65.8, *t*
19	11.9, *q*	11.8, *q*	12.7, *q*	11.9, *q*	12.9, *q*	12.7, *q*	11.8, *q*	11.7, *q*
20	17.6, *q*	18.1, *q*	18.1, *q*	18.2, *q*	18.2, *q*	18.0, *q*	18.2, *q*	17.8, *q*
OAc	172.2, *s*	171.8, *s*	171.7, *s*	171.8, *s*	171.8, *s*	170.4, *s*	171.8, *s*	171.8, *s*
	20.5, *q*	21.1, *q*	21.2, *q*	21.1, *q*	21.2, *q*	20.9, *q*	21.0, *q*	21.0, *q*
						170.3, *s*		
						20.9, *q*		
						169.8, *s*		
						20.7, *q*		

[a] $CDCl_3$ solution.
[b] *, These assignments may be reversed.

data from *neo*-clerodanes suggests that the composition of the substitution at C-4 (C-18) is important. Many of the *neo*-clerodanes with potent antifeedant activity have an epoxide substitution at C-4 but, to date, no *ent*-kauranes with an epoxide at this position have been tested for antifeedant activity against *S. littoralis*. However, sideroxol, one of the most potent *ent*-kaurane diterpenoids tested for antifeedant activity in the binary choice assay used in this study, has an epoxide at C-15–C-16. Although this compound showed potent antifeedant activity, this was against larvae of *Spodoptera frugiperda* (Feeding Index = 54 (4.3), $P < 0.05$) not *S. littoralis* (Feeding Index = 9 (15.9), (Bondì et al., 2000). In the present study no compounds had the C-15–C16 epoxide present in sideroxol, but the two compounds with an epoxide at C-16 (**5** and **6**) had antifeedant feeding activity against *S. littoralis* (Table 2). However, the antifeedant activity of the *ent*-kauranes cannot be totally determined by the presence of an epoxide, as other active antifeedant compounds (**8**, **14**, **18**, **20**, **21** and **26**) lacked this group. Whether the presence of a C-15–C-16 epoxide or addition of an epoxide at C-4 would enhance the activity of the *ent*-kaurenes justifies investigation.

A comparison of the activity of compounds **25** with **26**, the most potent antifeedant in the present study, illustrates how altering the position of substitutions on the molecule can alter its potency. For example, exchange of the acetyl group at C-18 (**25**) with the tigloyl group at C-3 (**26**) resulted in a significant increase in antifeedant activity. This would suggest that the position of these groups influence the ability of the molecule to stimulate receptors on the taste neurones of *S. littoralis*. The position of these groups alters the polarity of the molecule, as compound **26** is less polar

Table 1b
^{13}C NMR spectral data of compounds[a]

C	10	11	12	13	14	15	16	17	18
1	38.1, *t** [b]	38.4, *t**	38.4, *t**	38.1, *t**	38.3, *t**	37.8, *t**	38.3, *t**	38.0, *t*	37.7, *t**
2	23.0, *t*	23.0, *t*	23.0, *t*	23.1, *t*	23.0, *t*	22.9, *t*	23.1, *t*	23.1, *t*	23.0, *t*
3	73.5, *d*	74.2, *d*	74.1, *d*	73.5, *d*	75.1, *d*	74.2, *d*	74.7, *d*	74.0, *d*	74.3, *d*
4	40.4, *s*	40.5, *s*	40.5, *s*	40.4, *s*	40.7, *s*	40.3, *s*	40.7, *s*	40.3, *s*	40.2, *s*
5	39.8, *d*	38.0, *d*	38.0, *d*	39.9, *d*	38.1, *d*	39.4, *d*	38.1, *d*	39.6, *d*	39.2, *d*
6	24.3, *t*	27.0, *t*	27.0, *t*	24.4, *t*	27.0, *t*	23.9, *t*	27.0, *t*	24.1, *t*	23.9, *t*
7	78.9, *d*	76.7, *d*	76.6, *d*	78.8, *d*	76.5, *d*	79.7, *d*	76.5, *d*	79.4, *d*	79.9, *d*
8	46.8, *s*	47.9, *s*	47.9, *s*	46.8, *s*	47.9, *s*	47.0, *s*	47.9, *s*	47.1, *s*	46.7, *s*
9	50.9, *d*	50.0, *d*	50.0, *d*	51.0, *d*	49.9, *d*	51.3, *d*	50.0, *d*	51.4, *d*	50.8, *d*
10	38.6, *s*	38.6, *s*	38.6, *s*	38.7, *s*	38.6, *s*	38.5, *s*	38.6, *s*	38.6, *s*	38.4, *s*
11	17.7, *t*	17.8, *t*	17.8, *t*	17.8, *t*	17.8, *t*	17.8, *t*	17.8, *t*	17.9, *t*	17.7, *t*
12	33.2, *t*	33.5, *t*	33.5, *t*	33.3, *t*	33.4, *t*	33.1, *t*	33.4, *t*	33.2, *t*	33.0, *t*
13	43.5, *d*	43.5, *d*	43.6, *d*	43.5, *d*	43.5, *d*	43.3, *d*	43.5, *d*	43.4, *d*	43.2, *d*
14	37.7, *t**	37.7, *t**	37.7, *t**	37.7, *t**	37.7, *t**	37.7, *t**	37.7, *t**	38.0, *t*	37.6, *t**
15	44.9, *t*	44.9, *t*	45.0, *t*	45.0, *t*	44.9, *t*	45.1, *t*	44.9, *t*	45.3, *t*	45.0, *t*
16	154.0, *s*	154.8, *s*	154.8, *s*	154.1, *s*	154.7, *s*	153.6, *s*	154.8, *s*	153.9, *s*	153.6, *s*
17	103.8, *t*	103.6, *t*	103.6, *t*	103.9, *t*	103.5, *t*	103.9, *t*	103.5, *t*	103.9, *t*	103.8, *t*
18	65.0, *t*	64.6, *t*	64.7, *t*	65.2, *t*	65.0, *t*	64.6, *t*	65.0, *t*	64.8, *t*	64.5, *t*
19	12.9, *q*	13.0, *q*	13.0, *q*	12.9, *q*	13.2, *q*	13.1, *q*	13.2, *q*	13.3, *q*	12.9, *q*
20	18.0, *q*	17.8, *q*	17.8, *q*	18.0, *q*	17.8, *q*	17.8, *q*	17.8, *q*	17.9, *q*	17.7, *q*
OAc	170.5, *s*	171.5, *s*	171.4, *s*	170.5, *s*	171.4, *s*	170.5, *s*	171.5, *s*	170.7, *s*	170.4, *s*
	20.8, *q*	21.1, *q*	21.1, *q*	20.9, *q*	21.0, *q*	19.2, *q*	21.0, *q*	19.6, *q*	19.5, *q*
C=O′					165.7, *s*	165.6, *s*	165.5, *s*	165.5, *s*	157.9, *s*
C=O″						165.1, *s*		165.1, *s*	157.6, *s*
1′	173.8, *s*	173.8, *s*	173.0, *s*	173.1, *s*	130.5, *s*	130.6, *s*	123.0, *s*	123.1, *s*	
2′	27.9, *t*	27.9, *t*	36.6, *t*	36.7, *t*	129.4, *d*	129.4, *d*	131.4, *d*	131.5, *d*	144.7, *s*
3′	9.2, *q*	9.2, *q*	18.5, *t*	18.5, *t*	128.2, *d*	128.3, *d*	113.5, *d*	113.7, *d*	117.5, *d*
4′			13.6, *q*	13.8, *q*	132.8, *d*	132.8, *d*	163.2, *s*	163.3, *s*	111.7, *d*
5′					128.2, *d*	128.3, *d*	113.5, *d*	113.7, *d*	146.1, *d*
6′					129.4, *d*	129.4, *d*	131.4, *d*	131.5, *d*	
1″	173.5, *s*			172.8, *s*		130.3, *s*		122.8, *s*	
2″	27.9, *t*			36.5, *t*		129.3, *d*		131.4, *d*	144.5, *s*
3″	9.1, *q*			18.5, *t*		128.1, *d*		113.5, *d*	117.5, *d*
4″				13.7, *q*		132.7, *d*		163.2, *s*	111.5, *d*
5″						128.1, *d*		113.5, *d*	145.9, *d*
6″						129.3, *d*		131.4, *d*	
OCH_3′							55.3, *q*	55.4, *q*	
OCH_3″								55.3, *q*	

[a] $CDCl_3$ solution.
[b] *, These assignments may be reversed.

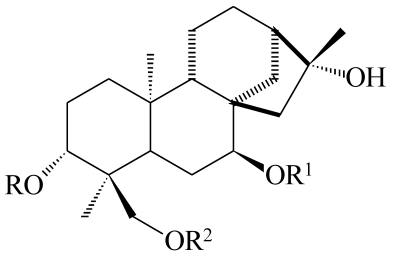
than **25**. Thus, whether changes in the polarity of the compound rather than the position or composition of specific functional groups on the molecule influence activity is not known.

To test the effect of compounds on insect feeding we usually test compounds dry on an inert substrate (glass–fibre discs). This avoids interactions between the test compound and compounds in leaf material but it also avoids problems associated with having to apply solvents to the insect mouth parts that we know could themselves modulate feeding by damaging insect taste neurones, such as high concentrations of ethanol. In our bioassay we apply a set aliquot (100 μl) of test compounds to the discs and always start with a dose of 100 ppm. The test compound is dissolved in an appropriate solvent, which will differ among compounds. An aliquot (100 μl) of the solvent is also applied to the control discs. The discs are dried before being presented to the insect. Although this type of bioassay is only suitable for a range of lepidopteran larvae and locusts it does enable us to provide quantitative comparative data about the potency of compounds for use in structure–activity relationship studies. However, the influence that compounds have on the acceptance or rejection of the discs by insects could depend on their solubility in the saliva produced during feeding or in the fluid that bathes their taste receptors. The composition of these fluids could be modified by the diet the insects are reared on, which is why we try and standardise the diet fed to our insects (Simmonds et al., 1992). As yet we known very little about the role these fluids have in modulating the ability of insects to detect compounds in their food and thus feeding behaviour of leaf chewing insects (Chapman and de Boer, 1995).

Table 1c
^{13}C NMR spectral data of compounds[a]

C	**19**	**20**	**21**	**22A >**	**22B <**	**23 >**	**24 <**	**25**	**26**
1	37.9, *t** [b]	37.7, *t**	38.0, *t**	38.3, *t**	38.3, *t**	37.8, *t*	37.8, *t*	38.0, *t**	38.1, *t**
2	22.8, *t*	22.7, *t*	23.0, *t*	22.9, *t*	22.8, *t*	22.9, *t*	22.9, *t*	23.1, *t*	23.1, *t*
3	75.3, *d*	75.0, *d*	75.5, *d*	74.3, *d*	74.4, *d*	74.2, *d*	73.7, *d*	73.9, *d*	74.4, *d*
4	40.5, *s*	40.3, *s*	40.6, *s*	40.7, *s*	40.6, *s*	40.3, *s*	40.3, *s*	40.5, *s*	40.5, *s*
5	39.7, *d*	39.5, *d*	39.9, *d*	38.0, *d*	38.0, *d*	39.9, *d*	39.5, *d*	39.7, *d*	40.2, *d*
6	23.9, *t*	23.7, *t*	24.0, *t*	26.9, *t*	26.9, *t*	24.1, *t*	23.9, *t*	24.1, *t*	24.3, *t*
7	81.2, *d*	80.9, *d*	81.4, *d*	76.6, *d*	76.6, *d*	79.7, *d*	79.4, *d*	79.2, *d*	79.4, *d*
8	46.8, *s*	46.7, *s*	47.0, *s*	47.9, *s*	47.9, *s*	48.0, *s*	47.9, *s*	47.1, *s*	47.2, *s*
9	51.3, *d*	51.1, *d*	51.5, *d*	50.0, *d*	50.0, *d*	48.3, *d*	48.3, *d*	51.3, *d*	51.3, *d*
10	38.5, *s*	38.4, *s*	38.6, *s*	38.6, *s*	38.6, *s*	38.5, *s*	38.5, *s*	38.7, *s*	38.7, *s*
11	17.8, *t*	17.6, *t*	17.9, *t*	17.8, *t*	17.8, *t*	17.6, *t*	17.6, *t*	17.9, *t*	17.9, *t*
12	33.0, *t*	32.9, *t*	33.1, *t*	33.4, *t*	33.4, *t*	26.8, *t*	26.8, *t*	33.3, *t*	33.3, *t*
13	43.2, *d*	43.0, *d*	43.3, *d*	43.5, *d*	43.5, *d*	51.9, *d*	51.9, *d*	43.5, *d*	43.5, *d*
14	37.6, *t**	37.5, *t**	37.8, *t**	37.6, *t**	37.6, *t**	35.9, *t*	35.8, *t*	37.9, *t**	37.9, *t**
15	45.1, *t*	45.0, *t*	45.2, *t*	44.9, *t*	44.9, *t*	54.2, *t*	54.2, *t*	45.3, *t*	45.3, *t*
16	153.2, *s*	153.1, *s*	153.2, *s*	154.8, *s*	154.8, *s*	78.6, *s*	78.7, *s*	154.1, *s*	154.1, *s*
17	104.1, *t*	104.0, *t*	104.3, *t*	103.6, *t*	103.6, *t*	24.3, *q*	24.3, *q*	103.8, *t*	103.8, *t*
18	64.5, *t*	64.3, *t*	64.7, *t*	64.5, *t*	64.6, *t*	65.1, *t*	65.0, *t*	65.2, *t*	65.3, *t*
19	13.0, *q*	12.9, *q*	13.1, *q*	13.0, *q*	13.0, *q*	12.8, *q*	13.1, *q*	13.2, *q*	12.9, *q*
20	17.8, *q*	17.6, *q*	17.9, *q*	17.8, *q*	17.8, *q*	18.2, *q*	18.1, *q*	18.0, *q*	18.1, *q*
OAc	170.0, *s*	169.9, *s*	170.1, *s*	171.4, *s*	171.4, *s*	170.5, *s*	170.7, *s*	170.7, *s*	170.5, *s*
	19.5, *q*	19.4, *q*	19.7, *q*	21.1, *q*	21.1, *q*	21.1, *q*	20.2, *q*	20.3, *q*	21.1, *q*
′C=O	163.5, *s*	163.9, *s*	163.9, *s*						
″C=O	163.1, *s*	163.4, *s*	163.5, *s*						
1′	132.3, *s*	134.2, *s*	136.0, *s*	173.6, *s*	173.7, *s*	166.7, *s*	167.3, *s*	167.4, *s*	167.0, *s*
2′	124.2, *d*	129.8, *d*	130.6, *d*	44.1, *d*	43.9, *d*	128.5, *s*	128.8, *s*	128.9, *s*	128.6, *s*
3′	148.2, *s*	132.1, *d*	123.6, *d*	137.2, *d*	137.1, *d*	136.2, *d*	137.0, *d*	136.8, *d*	136.3, *d*
4′	127.3, *d*	116.2, *s*	150.5, *s*	115.9, *t*	115.7, *t*	12.0, *q*	12.1, *q*	12.2, *q*	12.1, *q*
5′	129.7, *d*	132.1, *d*	123.6, *d*	16.5, *q*	16.7, *q*	14.3, *q*	14.3, *q*	14.3, *q*	14.3, *q*
6′	134.9, *d*	129.8, *d*	130.6, *d*						
1″	132.0, *s*	133.9, *s*	135.7, *s*			167.0, *s*	167.5, *s*	167.7, *s*	167.6, *s*
2″	124.2, *d*	129.7, *d*	130.5, *d*			129.0, *s*	129.1, *s*	129.2, *s*	129.2, *s*
3″	148.0, *s*	131.9, *d*	123.4, *d*			136.5, *d*	136.7, *d*	136.6, *d*	136.6, *d*
4″	127.1, *d*	116.0, *s*	150.4, *s*			12.0, *q*	12.1, *q*	12.0, *q*	12.0, *q*
5″	129.5, *d*	131.9, *d*	123.4, *d*			14.3, *q*	14.3, *q*	14.3, *q*	14.3, *q*
6″	134.9, *d*	129.7, *d*	130.5, *d*						
CN′		117.6, *s*							
CN″		117.5, *s*							

[a] $CDCl_3$ solution.
[b] *, These assignments may be reversed.



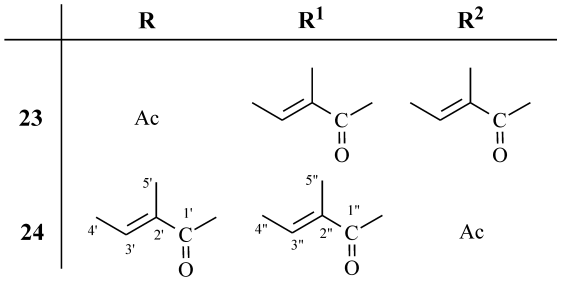


Table 2
Effect of compounds (100 ppm) on the feeding behaviour of final stadium larvae of *Spodoptera littoralis* ($n = 10$–15)

	Feeding Index[a]	
Compound	Mean	(SEM)
2	6.8	(12.91)
3	−9.4	(10.22)
4	19.5	(21.34)
5	39.0	(19.06)*
6	47.7	(25.06)*
7	22.6	(12.76)
8	42.4	(18.88)*
10	−37.5	(18.64)#
11	−1.6	(18.93)
13	0.0	(29.81)
14	27.7	(11.76)*
15	23.4	(12.54)
16	22.0	(14.07)
17	−6.0	(12.27)
18	48.3	(19.57)*
19	21.5	(13.32)
20	44.6	(11.55)*
21	34.0	(17.82)*
22	36.6	(25.91)
25	−15.5	(8.21)
26	74.1	(12.93)**

[a] Feeding Index ((C−T)/(C+T))%; significant antifeedant activity $*P<0.05$, $**P<0.01$; significant phagostimulant activity $\#P<0.05$, Wilcoxon matched-pairs test.

Despite these limitations in our knowledge the present study has shown that *ent*-kauranes can have potent antifeedant activity in a binary choice bioassay. For example, compound **26** at 100 ppm has elicited a higher Feeding Index than ajugarin I (Feeding Index = 43 (7.3), Simmonds et al., 1989) but not a high as Jodrellin B (Feeding Index = 100 (0.0), Cole et al., 1990) against *S. littoralis*. Further research is required to understand the molecular mechanism associated with the observed behavioural responses to these and other diterpenoids and whether different species of insects will show similar behavioural responses to these compounds.

4. Experimental

4.1. General experimental procedures

IR spectra (film) were registred on a Shimadzu FT–IR 8300 spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$ solution using a Bruker AC 250 E apparatus at 250 MHz and chemical shifts were reported with respect to residual $CHCl_3$ (δ 7.27). ^{13}C NMR spectra were recorded in $CDCl_3$ on the same apparatus at 62.7 MHz and chemical shifts were reported with respect to solvent signals (δ_{CDCl_3} 77.0). ^{13}C NMR assignments were determined by DEPT spectra. MS were recorded on a Finnigan TSQ70 instrument (70 eV, direct inlet). Melting points were determined on a Kofler block and are uncorrected. Merck Si gel no. 7734 (70–230 mesh) deactivated with 15% H_2O wt./v was used for column chromatography. An ozone generator Fischer 500 M has been used to perform ozonolysis. Linearol was isolated from the following species: *Sideritis akmanii, S. niveotomentosa, S. brevidens, S. rubriflora* and *S. gulendamii* (Bondì et al., 2000).

CH_2Cl_2 was dried by distillation over calcium hydride. Toluene was dried by distillation over molecular sieves (3 Å).

4.2. Reduction of linearol (2) to compounds 3 and 4

To a solution of **2** (50 mg) in MeOH (20 ml) was added Pd/C 10% (30 mg). This solution was subjected to a H_2 pressure (3 atm) in a Parr apparatus for 24 h. After filtration, the solvent was evaporated to give a mixture which was resolved by CC (silica gel, petrol–EtOAc 3:2 as eluent) yielding compound **3** (34 mg) and **4** (14 mg) in order of increasing polarity.

Compound **3**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3423, 2970, 2930, 2840, 1712, 1438, 1373, 1255, 1172, 1039, 1018, 979. EI–MS *m/z* (rel. int.): 364 $[M]^+$ (1), 346 $[M–H_2O]^+$ (5), 328 $[M–2(H_2O)]^+$ (14), 304 $[M–AcOH]^+$ (20), 286 $[M–AcOH–H_2O]^+$ (56), 268 $[M–AcOH–2(H_2O)]^+$ (100), 255 (42), 235 (83), 217 (36). ^{1}H NMR ($CDCl_3$): δ 3.52 (*dd*, 1H, $J = 9.0$ and 5.4 Hz, H-3β), 3.59 (*t*, 1H, $J = 3.0$ Hz, H-7α), 1.01 (*d*, 3H, $J = 6.4$ Hz, Me-17), 4.07 (*d*, 1H, $J = 11.6$ Hz, H_A-18), 3.96 (*d*, 1H, $J = 11.6$ Hz, H_B-18), 0.75 (*s*, 3H, Me-19), 1.05 (*s*, 3H, Me-20), 2.09 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

Compound **4**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3430, 2970, 2930, 2840, 1715, 1438, 1373, 1258, 1170, 1036, 1020, 985. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 346 $[M–H_2O]^+$ (2), 328 $[M–2(H_2O)]^+$ (2), 304 $[M–AcOH]^+$

(4), 286 $[M–AcOH–H_2O]^+$ (10), 268 $[M–AcOH–2(H_2O)]^+$ (10), 255 (100), 241 (30), 213 (21). ^1H NMR ($CDCl_3$): δ 4.91 (*dd*, 1H, $J=11.2$ and 5.4 Hz, H-3β), 3.60 (*t*, 1H, $J=3.0$ Hz, H-7α), 1.01 (*d*, 3H, $J=6.4$ Hz, Me-17), 3.33 (*d*, 1H, $J=12.4$ Hz, H_A-18), 2.99 (*d*, 1H, $J=12.4$ Hz, H_B-18), 0.67 (*s*, 3H, Me-19), 1.08 (*s*, 3H, Me-20), 2.07 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

4.3. Epoxidation of linearol (2) to compounds (5) and (6)

To a solution of **2** (250 mg) in CH_2Cl_2 (15 ml) was added MCPBA 70% (170 mg). After stirring overnight, the solution was extracted with a saturated aqueous solution of $NaHCO_3$ (3×10 ml) and washed with H_2O (3×10 ml). The organic layer was dried with dry Na_2SO_4 and evaporated under reduced pressure giving a residue which was purified by CC (silica gel, diethyl ether as eluent) yielding compounds **5** (140 mg) and **6** (92 mg).

Compound **5**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3480, 3267, 3040, 2931, 2866, 1714, 1384, 1371, 1255, 1049, 1018. EI–MS *m*/*z* (rel. int.): 378 $[M]^+$ (1), 360 $[M–H_2O]^+$ (5), 318 $[M–AcOH]^+$ (36), 300 $[M–H_2O–AcOH]^+$ (55), 282 $[M–AcOH–2(H_2O)]^+$ (97), 270 (100), 249 (96), 231 (41), 121 (85). ^1H NMR ($CDCl_3$): δ 3.54 (*dd*, 1H, $J=9.0$ and 5.2 Hz, H-3β), 3.73 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.91 (*d*, 1H, $J=4.5$ Hz, H_A-17), 2.82 (*d*, 1H, $J=4.5$ Hz, H_B-17), 4.06 (*d*, 1H, $J=11.8$ Hz, H_A-18), 3.99 (*d*, 1H, $J=11.8$ Hz, H_B-18), 0.77 (*s*, 3H, Me-19), 1.08 (*s*, 3H, Me-20), 2.08 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

Compound **6**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3388, 3040, 2933, 2868, 1724, 1369, 1250, 1053, 1024, 1004, 953, 736. EI–MS *m*/*z* (rel. int.): 378 $[M]^+$ (1), 360 $[M–H_2O]^+$ (2), 318 $[M–AcOH]^+$ (4), 300 $[M–AcOH–H_2O]^+$ (12), 282 $[M–AcOH–2(H_2O)]^+$ (22), 270 (100), 255 (40), 213 (11), 182 (12), 159 (12), 145 (19), 119 (22). ^1H NMR ($CDCl_3$): δ 4.90 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 3.72 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.91 (*d*, 1H, $J=4.6$ Hz, H_A-17), 2.79 (*d*, 1H, $J=4.6$ Hz, H_B-17), 3.29 (*d*, 1H, $J=12.6$ Hz, H_A-18), 2.96 (*d*, 1H, $J=12.6$ Hz, H_B-18), 0.67 (*s*, 3H, Me-19), 1.07 (*s*, 3H, Me-20), 2.05 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

4.4. Acetylation of compounds 5 and 6

Fifty milligrams of compounds **5** and **6** were treated separately with 5 ml of a mixture of pyridine–acetic anhydride (3:2) yielding, after evaporation under reduced pressure, the same compound **7** (98% yield).

Compound **7**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3040, 2939, 2868, 1734, 1732, 1371, 1246, 1030, 962, 902, 734. EI–MS *m*/*z* (rel. int.): 462 $[M]^+$ (1), 402 $[M–AcOH]^+$ (2), 342 $[M–2(AcOH)]^+$ (5), 329 (2), 300 (2), 282 $[M–3(AcOH)]^+$ (23), 269 (10), 119 (8), 95 (12), 43 (100). ^1H NMR ($CDCl_3$): δ 4.65 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 4.79 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.80 (*d*, 1H, $J=4.5$ Hz, H_A-17), 2.72 (*d*, 1H, $J=4.5$ Hz, H_B-17), 3.84 (*d*, 1H, $J=11.9$ Hz, H_A-18), 3.44 (*d*, 1H, $J=11.9$ Hz, H_B-18), 0.75 (*s*, 3H, Me-19), 1.04 (*s*, 3H, Me-20), 1.95 (*s*, 3H, OAc), 1.94 (*s*, 3H, OAc), 1.92 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

4.5. Ozonolysis of 2 to compounds 8 and 9

A current of O_2/O_3 (30 l/h) was bubbled into a solution of **2** (300 mg) in dry CH_2Cl_2 at −78 °C for 30 min. After evaporation of the solvent under reduced pressure, the residue was purified by CC (silica gel, gradient elution from petrol ether–AcOEt 2:3 to pure AcOEt) giving, in order of increasing polarity, 87 mg of compound **8** and 186 mg of compound **9**.

Compound **8**: white crystals, mp 155–158 °C. IR ν_{max} (film) cm^{-1}: 3433, 2935, 2870, 1732, 1720, 1371, 1251, 1047, 837, 734. EI–MS *m*/*z* (rel. int.): 364 $[M]^+$ (1), 346 $[M–H_2O]^+$ (4), 328 $[M–2(H_2O)]^+$ (5), 304 $[M–AcOH]^+$ (6), 286 $[M–AcOH–H_2O]^+$ (18), 268 $[M–AcOH–2(H_2O)]^+$ (10), 235 (15), 217 (11), 119 (13), 107 (20), 95 (26), 43 (100). ^1H NMR ($CDCl_3$): δ 3.51 (*dd*, 1H, $J=9.0$ and 5.2 Hz, H-3β), 3.66 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.37 (*m*, 1H, H-13), 2.10 (*br s*, 2H, H-15), 3.99 (*d*, 1H, $J=11.6$ Hz, H_A-18), 3.92 (*d*, 1H, $J=11.6$ Hz, H_B-18), 0.72 (*s*, 3H, Me-19), 1.06 (*s*, 3H, Me-20), 2.02 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

Compound **9**: white solid, mp 88–92. IR ν_{max} (film) cm^{-1}: 3445, 2937, 2870, 1735, 1372, 1248, 1040, 1005, 956, 734. EI–MS *m*/*z* (rel. int.): $[M]^+$ absent, 364 $[M–CH_2O_2]^+$ (1), 346 $[M–CH_2O_2–H_2O]^+$ (1), 304 $[M–CH_2O_2–AcOH]^+$ (3), 286 $[M–CH_2O_2–AcOH–H_2O]^+$ (5), 268 $[M–CH_2O_2–AcOH–2(H_2O)]^+$ (3), 245 (7), 227 (10), 145 (12), 119 (10), 107 (15), 91 (20), 43 (100). ^1H NMR ($CDCl_3$): δ 3.48 (*dd*, 1H, $J=9.0$ and 5.2 Hz, H-3β), 3.55 (*t*, 1H, $J=3.8$ Hz, H-7α), 5.01 (*br s*, 2H, H-17), 3.91 (*d*, 1H, $J=11.6$ Hz, H_A-18), 3.89 (*d*, 1H, $J=11.6$ Hz, H_B-18), 0.67 (*s*, 3H, Me-19), 0.98 (*s*, 3H, Me-20), 2.00 (*s*, 3H, OAc). ^{13}C NMR: Table 1a.

4.6. Transformation of compound 9 into compound 8

Twenty milligrams of ozonide **9** were dissolved in 10 ml of glacial acetic acid and added to zinc powder. After 30 min the conversion of **9** to **8** was complete. This was confirmed by TLC comparison with an authentic sample of **8**.

4.7. General esterification procedure

Linearol (150 mg) was solubilized in 10 ml of dry CH_2Cl_2 and added with 1 eq. of DMAP (56 mg), 25 eq. of TEA (1.5 ml) and the appropriate acyl chloride (4 eq.) at room temperature under argon atmosphere. After stirring overnight, the reaction was subjected to usual work-up adding H_2O and extracting with AcOEt; finally, the organic layer was dried over Na_2SO_4 and

evaporated under reduced pressure. Generally, the residue was purified by CC (silica gel, mixtures of petrol–AcOEt as eluent). This procedure gave the following ester derivatives:

Compounds **10** and **11**. Treatment of **2** with propanoyl chloride gave a mixture of two compounds which were separated by CC (silica gel, petrol ether–AcOEt 4:1 as eluent) giving 125 mg of **10** and 47 mg of **11**.

Compound **10**: amorphous solid. IR ν_{max} (film) cm^{-1}: 2937, 2858, 1732, 1660, 1462, 1371, 1230, 1195, 1178, 1150, 1070, 1041, 1024, 875. EI–MS *m/z* (rel. int.): 474 $[M]^+$ (1), 400 $[M–CH_3CH_2COOH]^+$ (9), 340 $[M–CH_3CH_2COOH–AcOH)]^+$ (2), 326 $[M–2(CH_3CH_2COOH)]^+$ (93), 283 (2), 266 $[M–2(CH_3CH_2COOH)–AcOH]^+$ (82), 253 (28), 185 (22), 119 (31), 105 (28), 43 (100). ^1H NMR ($CDCl_3$): δ 4.77 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 4.77 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.70 (*m*, 1H, H-13), 2.17 (*dt*, 1H, $J=17$ and 2.2 Hz, H_A-15), 2.13 (*dt*, 1H, $J=17$ and 2.2 Hz, H_B-15), 4.82 (*br s*, 1H, H_A-17), 4.81 (*br s*, 1H, H_B-17), 3.92 (*d*, 1H, $J=11.8$ Hz, H_A-18), 3.53 (*d*, 1H, $J=11.8$ Hz, H_B-18), 0.83 (*s*, 3H, Me-19), 1.09 (*s*, 3H, Me-20), 2.02 (*s*, 3H, OAc), 2.31 (*q*, 2H, $J=7.5$ Hz, H-2′), 1.16 (*t*, 3H, $J=7.5$ Hz, H-3′), 2.30 (*q*, 2H, $J=7.5$ Hz, H-2″), 1.11 (*t*, 3H, $J=7.5$ Hz, H-3″). ^{13}C NMR: Table 1b.

Compound **11**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3435, 2937, 2858, 1730, 1660, 1462, 1423, 1371, 1230, 1180, 1041, 1024. EI–MS *m/z* (rel. int.): 418 $[M]^+$ (1), 400 $[M–H_2O]^+$ (2), 344 $[M–CH_3CH_2COOH)]^+$ (2), 326 $[M–CH_3CH_2COOH–H_2O]^+$ (37), 284 $[M–CH_3CH_2COOH–AcOH]^+$ (10), 266 $[M–CH_3CH_2COOH–AcOH–H_2O]^+$ (42), 251 (23), 149 (32), 121 (35), 93 (35), 43 (100). ^1H NMR ($CDCl_3$): δ 4.86 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 3.60 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.69 (*m*, 1H, H-13), 2.26 (*br s*, 2H, H-15), 4.83 (*br s*, 1H, H_A-17), 4.80 (*br s*, 1H, H_B-17), 4.08 (*d*, 1H, $J=11.6$ Hz, H_A-18), 3.54 (*d*, 1H, $J=11.6$ Hz, H_B-18), 0.84 (*s*, 3H, Me-19), 1.09 (*s*, 3H, Me-20), 2.07 (*s*, 3H, OAc), 2.32 (*q*, 2H, $J=7.5$ Hz, H-2′), 1.14 (*t*, 3H, $J=7.5$ Hz, H-3′). ^{13}C NMR: Table 1b.

Compounds **12** and **13**. Treatment of **2** with butanoyl chloride gave a mixture of two compounds which were separated by CC (silica gel, petrol ether–AcOEt 4:1 as eluent) giving 50 mg of **12** and 120 mg of **13**.

Compound **12**: amorphous solid. IR ν_{max} (film) cm^{-1}: 3440, 2937, 2858, 1725, 1660, 1458, 1425, 1367, 1235, 1185, 1020. EI–MS *m/z* (rel. int.): 432 $[M]^+$ (1), 414 $[M–H_2O]^+$ (1), 344 $[M–CH_3CH_2CH_2COOH)]^+$ (2), 326 $[M–CH_3CH_2CH_2COOH–H_2O]^+$ (30), 284 $[M–CH_3CH_2CH_2COOH–AcOH]^+$ (7), 266 $[M–CH_3CH_2CH_2COOH–AcOH\text{-}H_2O]^+$ (50), 149 (42), 121 (30), 93 (20), 71 (33), 43 (100). ^1H NMR ($CDCl_3$): δ 4.87 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 3.60 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.69 (*m*, 1H, H-13), 2.27 (*br s*, 2H, H-15), 4.83 (*br s*, 1H, H_A-17), 4.80 (*br s*, 1H, H_B-17), 4.08 (*d*, 1H, $J=11.7$ Hz, H_A-18), 3.53 (*d*, 1H, $J=11.7$ Hz, H_B-18), 0.83 (*s*, 3H, Me-19), 1.09 (*s*, 3H, Me-20), 2.07 (*s*, 3H, OAc), 2.27 (*t*, 2H, $J=7.5$ Hz, H-2′), 0.95 (*t*, 3H, $J=7.5$ Hz, H-4′). ^{13}C NMR: Table 1b.

Compound **13**: amorphous solid. IR ν_{max} (film) cm^{-1}: 2931, 2875, 2856, 1732, 1658, 1460, 1371, 1232, 1193, 1174, 1041, 873, 754. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 414 $[M–CH_3CH_2CH_2COOH]^+$ (6), 354 $[M–CH_3CH_2CH_2COOH–AcOH)]^+$ (2), 326 $[M–2(CH_3CH_2CH_2COOH)]^+$ (82), 266 $[M–2(CH_3CH_2CH_2COOH)–AcOH]^+$ (100), 253 (29), 185 (20). ^1H NMR ($CDCl_3$): δ 4.78 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 4.77 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.71 (*m*, 1H, H-13), 2.16 (*dt*, 1H, $J=17$ and 2.2 Hz, H_A-15), 2.12 (*dt*, 1H, $J=17$ and 2.2 Hz, H_B-15), 4.82 (*br s*, 1H, H_A-17), 4.81 (*br s*, 1H, H_B-17), 3.90 (*d*, 1H, $J=11.7$ Hz, H_A-18), 3.54 (*d*, 1H, $J=11.7$ Hz, H_B-18), 0.83 (*s*, 3H, Me-19), 1.11 (*s*, 3H, Me-20), 2.03 (*s*, 3H, OAc), 2.28 (*t*, 4H, $J=7.3$ Hz, H-2′ and H-2″), 0.97 (*t*, 3H, $J=7.5$ Hz, H-4′), 0.93 (*t*, 3H, $J=7.5$ Hz, H-4″). ^{13}C NMR: Table 1b.

Compounds **14** and **15**. Treatment of **2** with benzoyl chloride gave a mixture of two compounds which were separated by CC (silica gel, petrol ether–AcOEt 4:1, petrol–AcOEt 3:2 as eluent) giving 37 mg of **14** and 180 mg of **15**.

Compound **14**: white crystals, mp 176–180 °C. IR ν_{max} (film) cm^{-1}: 3527, 2933, 2858, 1743, 1716, 1655, 1600, 1450, 1273, 1250, 1176, 1115, 943, 736, 711. EI–MS *m/z* (rel. int.): 466 $[M]^+$ (1), 448 $[M–H_2O]^+$ (2), 344 $[M–C_6H_5COOH]^+$ (2), 326 $[M–C_6H_5COOH–H_2O]^+$ (35), 284 $[M–C_6H_5COOH–AcOH]^+$ (10), 266 $[M–C_6H_5COOH–AcOH–H_2O]^+$ (37), 176 (22), 149 (47), 105 (93), 84 (100). ^1H NMR ($CDCl_3$): δ 5.09 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 3.59 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.67 (*m*, 1H, H-13), 2.26 (*br s*, 2H, H-15), 4.81 (*br s*, 1H, H_A-17), 4.79 (*br s*, 1H, H_B-17), 4.17 (*d*, 1H, $J=11.7$ Hz, H_A-18), 3.56 (*d*, 1H, $J=11.7$ Hz, H_B-18), 0.98 (*s*, 3H, Me-19), 1.11 (*s*, 3H, Me-20), 2.03 (*s*, 3H, OAc), 8.00 (*dd*, 2H, $J=7.0$ and 1.6 Hz, H-2′ and H-6′), 7.41 (*td*, 2H, $J=7.0$ and 1.6 Hz, H-3′ and H-5′), 7.51 (*tt*, 1H, $J=7.0$ and 1.6 Hz, H-4′). ^{13}C NMR: Table 1b.

Compound **15**: white crystals, mp 175–178 °C. IR ν_{max} (film) cm^{-1}: 3060, 2933, 2860, 1743, 1716, 1658, 1600, 1450, 1276, 1249, 1176, 1114, 879, 738, 711. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 448 $[M–C_6H_5COOH]^+$ (3), 388 $[M–C_6H_5COOH–AcOH]^+$ (2), 326 $[M–2(C_6H_5COOH)]^+$ (58), 266 $[M–2(C_5H_6COOH)–AcOH]^+$ (70), 251 (32), 185 (32), 119 (18), 105 (100). ^1H NMR ($CDCl_3$): δ 5.02 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 5.02 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.75 (*m*, 1H, H-13), 2.35 (*dt*, 1H, $J=17$ and 2.2 Hz, H_A-15), 2.20 (*dt*, 1H, $J=17$ and 2.2 Hz, H_B-15), 4.83 (*br s*, 1H, H_A-17), 4.76 (*br s*, 1H, H_B-17), 3.80 (*d*, 1H, $J=11.7$ Hz, H_A-18), 3.60 (*d*, 1H, $J=11.7$ Hz, H_B-18), 0.99 (*s*, 3H, Me-19), 1.21 (*s*, 3H, Me-20), 1.12 (*s*, 3H, OAc), 8.05 (*dd*, 2H, $J=7.0$ and 1.6 Hz, H-2′ and H-6′), 7.46 (*td*, 2H, $J=7.0$ and 1.6 Hz, H-3′ and H-5′), 7.54 (*tt*, 1H, $J=7.0$ and 1.6 Hz, H-4′), 7.96 (*dd*, 2H, $J=7.0$ and 1.6 Hz, H-2″ and H-6″), 7.40 (*td*, 2H, $J=7.0$ and 1.6 Hz, H-3″ and H-5″), 7.51 (*tt*, 1H, $J=7.0$ and 1.6 Hz, H-4″). ^{13}C NMR: Table 1b.

Compounds **16** and **17**. Treatment of **2** with 4-methoxybenzoyl chloride gave a mixture of two compounds which were separated by CC (silica gel, petrol ether–AcOEt 4:1, petrol ether–AcOEt 3:2 as eluent) giving 60 mg of **16** and 175 mg of **17**.

Compound **16**: white crystals, mp 187–190 °C. IR ν_{max} (film) cm^{-1}: 3508, 2931, 2856, 1740, 1700, 1654, 1606, 1579, 1512, 1317, 1270, 1256, 1167, 1101, 1029, 869, 771, 736. EI–MS *m/z* (rel. int.): 496 $[M]^+$ (2), 478 $[M–H_2O]^+$ (6), 418 $[M–H_2O–AcOH]^+$ (16), 344 $[M–CH_3OC_6H_5COOH]^+$ (3), 326 $[M–CH_3OC_6H_5COOH–H_2O]^+$ (32), 284 $[M–CH_3OC_6H_5COOH–AcOH]^+$ (18), 266 $[M–CH_3OC_6H_5COOH–AcOH–H_2O]^+$ (80), 253 (60), 135 (100). ^1H NMR ($CDCl_3$): δ 5.06 (*dd*, 1H, *J* = 11.2 and 5.2 Hz, H-3β), 3.58 (*t*, 1H, *J* = 3.8 Hz, H-7α), 2.67 (*m*, 1H, H-13), 2.26 (*br s*, 2H, H-15), 4.81 (*br s*, 1H, H_A-17), 4.78 (*br s*, 1H, H_B-17), 4.16 (*d*, 1H, *J* = 11.7 Hz, H_A-18), 3.56 (*d*, 1H, *J* = 11.7 Hz, H_B-18), 0.97 (*s*, 3H, Me-19), 1.14 (*s*, 3H, Me-20), 2.03 (*s*, 3H, OAc), 7.95 (*d*, 2H, *J* = 6.8 Hz, H-2′ and H-6′), 6.90 (*d*, 2H, *J* = 6.8 Hz, H-3′ and H-5′), 3.83 (*s*, 3H, OME′). ^{13}C NMR: Table 1b.

Compound **17**: white crystals, mp 83–86 °C. IR ν_{max} (film) cm^{-1}: 3064, 2935, 2860, 1743, 1708, 1658, 1606, 1581, 1512, 1461, 1317, 1270, 1257, 1166, 1101, 1029, 877, 771, 736. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 478 $[M–CH_3OC_6H_5COOH]^+$ (3), 326 $[M–2(CH_3OC_6H_5COOH)]^+$ (76), 266 $[M–2(CH_3OC_5H_6COOH)–AcOH]^+$ (100), 251 (28), 135 (96). ^1H NMR ($CDCl_3$): &*delta*; 4.97 (*dd*, 1H, *J* = 11.2 and 5.2 Hz, H-3β), 4.97 (*t*, 1H, *J* = 3.8 Hz, H-7α), 2.73 (*m*, 1H, H-13), 2.32 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_A-15), 2.18 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_B-15), 4.82 (*br s*, 1H, H_A-17), 4.74 (*br s*, 1H, H_B-17), 3.76 (*d*, 1H, *J* = 11.7 Hz, H_A-18), 3.60 (*d*, 1H, *J* = 11.7 Hz, H_B-18), 0.97 (*s*, 3H, Me-19), 1.20 (*s*, 3H, Me-20), 1.20 (*s*, 3H, OAc), 8.00 (*d*, 2H, *J* = 6.8 Hz, H-2′ and H-6′), 6.94 (*d*, 2H, *J* = 6.8 Hz, H-3′ and H-5′), 7.91 (*d*, 2H, *J* = 6.8 Hz, H-2″ and H-6″), 6.87 (*d*, 2H, *J* = 6.8 Hz, H-3″ and H-5″), 3.84 (*s*, 3H, OME′), 3.82 (*s*, 3H, OME″). ^{13}C NMR: Table 1b.

Compound **18**. Treatment of **2** with 2-furoyl chloride gave 187 mg of **18**. Amorphous solid. IR ν_{max} (film) cm^{-1}: 3124, 3064, 2937, 2862, 1744, 1714, 1660, 1579, 1569, 1471, 1394, 1299, 1232, 1180, 1120, 1041, 1012, 975, 885, 763, 736, 702. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 438 $[M–C_4H_3OCOOH]^+$ (2), 326 $[M–2(C_4H_3OCOOH)]^+$ (50), 266 $[M–2(C_4H_3OCOOH)–AcOH]^+$ (52), 251 (16), 185 (14), 95 (100). ^1H NMR ($CDCl_3$): δ 4.95 (*dd*, 1H, *J* = 11.2 and 5.2 Hz, H-3β), 4.91 (*t*, 1H, *J* = 3.8 Hz, H-7α), 2.67 (*m*, 1H, H-13), 2.25 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_A-15), 2.10 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_B-15), 4.77 (*br s*, 1H, H_A-17), 4.70 (*br s*, 1H, H_B-17), 3.77 (*d*, 1H, *J* = 11.7 Hz, H_A-18), 3.54 (*d*, 1H, *J* = 11.7 Hz, H_B-18), 0.88 (*s*, 3H, Me-19), 1.11 (*s*, 3H, Me-20), 1.37 (*s*, 3H, OAc), 7.13 (*m*, 1H, H-3′), 6.47 (*m*, 1H, H-4′), 7.54 (*m*, 1H, H-5′), 7.04 (*m*, 1H, H-3″), 6.42 (*m*, 1H, H-4″), 7.49 (*m*, 1H, H-5″). ^{13}C NMR: Table 1b.

Compound **19**. Treatment of **2** with 3-nitrobenzoyl chloride gave 220 mg of **19**. Amorphous solid. IR ν_{max} (film) cm^{-1}: 3085, 3064, 2937, 2864, 1747, 1732, 1658, 1616, 1537, 1479, 1440, 1260, 1234, 1136, 1041, 837, 775, 719. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 493 $[M–NO_2C_6H_4COOH]^+$ (3), 433 $[M–NO_2C_6H_4COOH–AcOH]^+$ (3), 326 $[M–2(NO_2C_6H_4COOH)]^+$ (53), 266 $[M–2(NO_2C_6H_4COOH)–AcOH]^+$ (100), 251 (45), 150 (67), 119 (24), 83 (86). ^1H NMR ($CDCl_3$): δ 5.00 (*dd*, 1H, *J* = 11.2 and 5.2 Hz, H-3β), 5.03 (*t*, 1H, *J* = 3.8 Hz, H-7α), 2.72 (*m*, 1H, H-13), 2.33 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_A-15), 2.17 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_B-15), 4.80 (*br s*, 1H, H_A-17), 4.73 (*br s*, 1H, H_B-17), 3.86 (*d*, 1H, *J* = 11.7 Hz, H_A-18), 3.54 (*d*, 1H, *J* = 11.7 Hz, H_B-18), 1.00 (*s*, 3H, Me-19), 1.20 (*s*, 3H, Me-20), 1.17 (*s*, 3H, OAc), 8.81 (*br s*, 1H, H-2′), 8.41 (*br d*, 1H, *J* = 7.5, H-4′), 7.70 (*t*, 1H, *J* = 7.5, H-5′), 8.37 (*br d*, 1H, *J* = 7.5, H-6′), 8.70 (*br s*, 1H, H-2″), 8.34 (*br d*, 1H, *J* = 7.5, H-4″), 7.60 (*t*, 1H, *J* = 7.5, H-5″), 8.24 (*br d*, 1H, *J* = 7.5, H-6″). ^{13}C NMR: Table 1c.

Compound **20**. Treatment of **2** with 4-cyanobenzoyl chloride gave 232 mg of **20**. Amorphous solid. IR ν_{max} (film) cm^{-1}: 3064, 2937, 2862, 2231, 1744, 1716, 1658, 1610, 1569, 1309, 1276, 1234, 1176, 1041, 1015, 862, 767, 736. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 326 $[M–2(NCC_6H_4COOH)]^+$ (24), 266 $[M–2(NCC_6H_4COOH)–AcOH]^+$ (44), 251 (20), 185 (16), 130 (100). ^1H NMR ($CDCl_3$): δ 4.96 (*dd*, 1H, *J* = 11.2 and 5.2 Hz, H-3β), 4.98 (*t*, 1H, *J* = 3.8 Hz, H-7α), 2.70 (*m*, 1H, H-13), 2.28 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_A-15), 2.13 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_B-15), 4.78 (*br s*, 1H, H_A-17), 4.71 (*br s*, 1H, H_B-17), 3.79 (*d*, 1H, *J* = 11.7 Hz, H_A-18), 3.52 (*d*, 1H, *J* = 11.7 Hz, H_B-18), 0.94 (*s*, 3H, Me-19), 1.15 (*s*, 3H, Me-20), 1.14 (*s*, 3H, OAc), 8.12 (*br d*, 2H, *J* = 6.8 Hz, H-2′ and H-6′), 7.75 (*br d*, 2H, *J* = 6.8 Hz, H-3′ and H-5′), 8.00 (*br d*, 2H, *J* = 6.8 Hz, H-2″ and H-6″), 7.66 (*br d*, 2H, *J* = 6.8 Hz, H-3″ and H-5″). ^{13}C NMR: Table 1c.

Compound **21**. Treatment of **2** with 4-nitrobenzoyl chloride gave 260 mg of **21**. Amorphous solid. IR ν_{max} (film) cm^{-1}: 3057, 2935, 2862, 1743, 1722, 1656, 1606, 1529, 1344, 1276, 1234, 1118, 1103, 1041, 1014, 873, 842, 785, 736, 719. EI–MS *m/z* (rel. int.): $[M]^+$ absent, 493 $[M–NO_2C_6H_4COOH]^+$ (1), 433 $[M–NO_2C_6H_4COOH–AcOH]^+$ (2), 326 $[M–2(NO_2C_6H_4COOH)]^+$ (40), 266 $[M–2(NO_2C_6H_4COOH)–AcOH]^+$ (100), 251 (57), 185 (35), 150 (82). ^1H NMR ($CDCl_3$): δ 5.02 (*dd*, 1H, *J* = 11.2 and 5.2 Hz, H-3β), 5.04 (*t*, 1H, *J* = 3.8 Hz, H-7α), 2.77 (*m*, 1H, H-13), 2.33 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_A-15), 2.20 (*dt*, 1H, *J* = 17 and 2.2 Hz, H_B-15), 4.85 (*br s*, 1H, H_A-17), 4.77 (*br s*, 1H, H_B-17), 3.86 (*d*, 1H, *J* = 11.7 Hz, H_A-18), 3.59 (*d*, 1H, *J* = 11.7 Hz, H_B-18), 1.00 (*s*, 3H, Me-19), 1.22 (*s*, 3H, Me-20), 1.21 (*s*, 3H, OAc), 8.23 (*br d*, 2H, *J* = 6.8 Hz, H-2′ and H-6′), 8.33 (*br d*, 2H, *J* = 6.8 Hz, H-3′ and H-5′), 8.11 (*br d*, 2H, *J* = 6.8 Hz, H-2″ and H-6″), 8.24 (*br d*, 2H, *J* = 6.8 Hz, H-3″ and H-5″). ^{13}C NMR: Table 1c.

Compound **22**. Treatment of **2** with tigloyl chloride gave 92 mg of **22** an unsemixture of two compounds in a 3:2 ratio. Amorphous solid. IR ν_{max} (film) cm^{-1}: 3531, 2981, 2935, 2877, 1732, 1656, 1639, 1458, 1371, 1250, 1193, 1049, 995, 977, 873, 756. EI–MS *m/z* (rel. int.): 444 $[M]^+$ (1), 426 $[M–H_2O]^+$ (3), 344 $[M–C_4H_7COOH]^+$ (8), 326 $[M–C_4H_7COOH\text{-}H_2O]^+$ (92), 284 $[M–C_4H_7COOH–AcOH]^+$ (23), 266 $[M–C_4H_7COOH–H_2O–AcOH]^+$ (100), 253 (32), 223 (14), 83 (40). ^1H NMR ($CDCl_3$): δ 4.86 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 3.57 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.66 (*m*, 1H, H-13), 2.25 (*br s*, 2H, H-15), 4.80 (*br s*, 1H, H_A-17), 4.77 (*br s*, 1H, H_B-17), 4.07 (*d*, 1H, $J=11.6$ Hz, H_A-18), 3.45 (*d*, 0.6H, $J=11.6$ Hz, H_B-18, 22A epimer), 3.49 (*d*, 0.4H, $J=11.6$ Hz, H_B-18, 22B epimer), 0.81 (*s*, 3H, Me-19), 1.07 (*s*, 3H, Me-20), 2.04 (*s*, 3H, OAc), 3.11 (*br quint*, 1H, $J=7.0$ Hz, H-2′), 5.87 (*m*, 1H, H-3′), 5.08 (*br d*, 1H, $J=10.0$ Hz, H_A-4′), 5.12 (*br d*, 1H, $J=17.2$ Hz, H_B-4′), 1.24 (*d*, 1.8H, $J=7.0$ Hz, Me-5, 22A epimer), 1.25 (*d*, 1.2H, $J=7.0$ Hz, Me-5′, 22B epimer). ^{13}C NMR: Table 1c.

Compounds **23** and **24**. Treatment of **2** (150 mg) with 287 mg of tigloyl chloride in refluxing toluene for 30 min gave 140 mg of an unresolvable mixture of **23** and **24** in a 55:45 ratio. Amorphous solid. IR ν_{max} (film) cm^{-1}: 3435, 2935, 2868, 1733, 1708, 1652, 1380, 1270, 1139, 1072, 1028, 732. EI–MS *m/z* (rel. int.): 544 $[M]^+$ (1), 526 $[M–H_2O]^+$ (3), 426 $[M–H_2O–C_4H_7COOH]^+$ (4), 366 $[M–H_2O–C_4H_7COOH–AcOH]^+$ (15), 344 $[M–2(C_4H_7COOH)]^+$ (7), 326 $[M–H_2O–2(C_4H_7COOH)]^+$ (33), 284 $[M–2(C_4H_7COOH)–AcOH]^+$ (97), 266 $[M–2(C_4H_7COOH)–H_2O–AcOH]^+$ (100), 225 (20). ^1H NMR ($CDCl_3$): δ 4.88 (*dd*, 0.55H, $J=11.2$ and 5.2 Hz, H-3β of **23**), 4.82 (*dd*, 0.45H, $J=11.2$ and 5.2 Hz, H-3β of **24**), 4.78 (*t*, 1H, $J=3.8$ Hz, H-7α), 1.35 (*s*, 3H, Me-17), 3.83 (*d*, 0.55H, $J=12.0$ Hz, H_A-18 of **23**), 3.78 (*d*, 0.45H, $J=12.0$ Hz, H_A-18 of **24**), 3.58 (*d*, 0.55H, $J=12.0$ Hz, H_B-18 of **23**), 3.54 (*d*, 0.45H, $J=12.0$ Hz, H_B-18 of **24**), 0.84 (*s*, 1.35H, Me-19 of **24**), 0.86 (*s*, 1.65H, Me-19 of **23**), 1.13 (*s*, 3H, Me-20), 1.99 (*s*, 1.65H, OAc of **23**), 1.82 (*s*, 1.35H, OAc of **24**), 6.69 (*qq*, 1.55H, $J=7.0$ and 1.0 Hz, H-3′ and H-3″ of **23** and H-3″ of **24**), 6.82 (*qq*, 0.45H, $J=7.0$ and 1.0 Hz, H-3′ of **24**), 1.77 (*br d*, 6H, $J=7.0$ Hz, Me-4′ and Me-4″), 1.71 (*br s*, 6H, Me-5′ and Me-5″). ^{13}C NMR: Table 1c.

Compounds **25** and **26**. Treatment of **2** (150 mg) with 287 mg of tigloyl chloride and 1.5 ml of TEA in refluxing toluene for 24 h gave a mixture of two compounds which were separated by CC (silica gel, gradient elution from petrol ether to petrol ether–AcOEt 3:2) giving 50 mg of **25** and 70 mg of **26**.

Compound **25**. Amorphous solid. IR ν_{max} (film) cm^{-1}: 2931, 2858, 1745, 1707, 1653, 1630, 1265, 1236, 1137, 1074, 1021, 732. EI–MS *m/z* (rel. int.): 526 $[M]^+$ (1), 426 $[M–C_4H_7COOH]^+$ (8), 366 $[M–C_4H_7COOH–AcOH]^+$ (10), 326 $[M–2(C_4H_7COOH)]^+$ (90), 266 $[M–2(C_4H_7COOH)–AcOH]^+$ (100), 251 (26), 226 (15). ^1H NMR ($CDCl_3$): δ 4.80 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 4.77 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.71 (*m*, 1H, H-13), 2.22 (*dt*, 1H, $J=17$ and 2.2 Hz, H_A-15), 2.10 (*dt*, 1H, $J=17$ and 2.2 Hz, H_B-15), 4.81 (*br s*, 1H, H_A-17), 4.76 (*br s*, 1H, H_B-17), 3.78 (*d*, 1H, $J=11.8$ Hz, H_A-18), 3.56 (*d*, 1H, $J=11.8$ Hz, H_B-18), 0.87 (*s*, 3H, Me-19), 1.13 (*s*, 3H, Me-20), 1.84 (*s*, 3H, OAc), 6.86 (*qq*, 1H, $J=7.0$ and 1.0 Hz, H-3′), 6.79 (*qq*, 1H, $J=7.0$ and 1.0 Hz, H-3″), 1.76 (*br d*, 6H, $J=7.1$ Hz, Me-4′ and Me-4″), 1.78 (*br s*, 6H, Me-5′ and Me-5″). ^{13}C NMR: Table 1c.

Compound **26**. Amorphous solid. IR ν_{max} (film) cm^{-1}: 2925, 2856, 1745, 1709, 1653, 1630, 1269, 1238, 1140, 1074, 1026, 756, 732. EI–MS *m/z* (rel. int.): 526 $[M]^+$ (1), 426 $[M–C_4H_7COOH]^+$ (10), 366 $[M–C_4H_7COOH–AcOH]^+$ (20), 326 $[M–2(C_4H_7COOH)]^+$ (80), 266 $[M–2(C_4H_7COOH)–AcOH]^+$ (100), 251 (30), 226 (20). ^1H NMR ($CDCl_3$): δ 4.89 (*dd*, 1H, $J=11.2$ and 5.2 Hz, H-3β), 4.77 (*t*, 1H, $J=3.8$ Hz, H-7α), 2.71 (*m*, 1H, H-13), 2.21 (*dt*, 1H, $J=17$ and 2.2 Hz, H_A-15), 2.12 (*dt*, 1H, $J=17$ and 2.2 Hz, H_B-15), 4.81 (*br s*, 1H, H_A-17), 4.76 (*br s*, 1H, H_B-17), 3.87 (*d*, 1H, $J=12.0$ Hz, H_A-18), 3.59 (*d*, 1H, $J=12.0$ Hz, H_B-18), 0.87 (*s*, 3H, Me-19), 1.14 (*s*, 3H, Me-20), 2.01 (*s*, 3H, OAc), 6.73 (*qq*, 2H, $J=7.0$ and 1.0 Hz, H-3′ and H-3″), 1.75 (*br d*, 6H, $J=7.1$ Hz, Me-4′ and Me-4″), 1.74 (*br s*, 6H, Me-5′ and Me-5″). ^{13}C NMR: Table 1c.

4.8. Antifeedant bioassay

Compounds were exposed to final stadium larvae of *Spodoptera littoralis* in a binary choice test using glass-fibre discs (Simmonds et al., 1990). All compounds were tested at 100 ppm as this is the dose used to compare activity among compounds (Rodríguez et al., 1999; Bondì et al., 2000). The majority of compounds were tested against insects from 2–3 generations, therefore, each compound was tested against either 10 or 15 insects. All discs were treated with 100 μl sucrose (0.05 M), and then the treatment discs were treated with 100 μl of one of the test compounds. The control discs were treated with an aliquot (100 μl) of the solvent used to dissolve the test compound. Larvae were taken from a colony fed on a wheat-germ based diet (Simmonds et al., 1992). Larvae 24–36 h into the final stadium were removed from food 2–3 h prior to the bioassay. These larvae were placed individually in Petri dishes (9.1 cm diam.) with a pair of discs: one treatment (T) and one control (C) disc. The discs were dried and weighed before and after being exposed to the insects. The amount eaten of each disc was used to calculate a Feeding Index $[(C-T)/(C+T)]\%$. A positive Index indicates an antifeedant and a negative Index a phagostimulant. The data did not differ significantly among generations and were pooled for analysis using the Wilcoxon matched-pairs test.

Acknowledgements

This paper was supported by MURST research funds. We thank Mrs. M. Yule and Dr. P. Green for support in rearing the insects. The insects bioassays were carried out at RBG Kew under a MAFF, Plant Health Great Britain licence.

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