

Stereoselective Michael Addition of 6-Amino-1,3-dimethyl-2,4-pyrimidinedione to the Exocyclic Methylene of Three Sesquiterpene Lactones. ^1H and ^{13}C NMR Evidence of a New C-C Bond and Lactam Formation

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The stereoselective addition of the pyrimidine derivative **1** to the exocyclic methylene of the α,β unsaturated dehydrocostus lactone **2**, Ivalin acetate **3** and Zaluzanin A diacetate **4**, was achieved resulting in a new C-C bond formation. In the cases of compounds **3** and **4**, after the addition, the lactone was cleaved followed by reclosure into a lactam ring system.

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Introduction.

Recently, a novel class of broad spectrum antiviral agents has been identified, which exhibits a potent and selective activity against adeno herpes-pox retrovirus. These compounds contain a pyrimidine base linked to a phosphonyl-alkyl group [1]. In addition, some analogs of the antibiotic oxetanocin, have been reported to possess antiviral activity [2]. These compounds involve carbocyclic moieties and a purine (adenine, 2,6-diaminopurine) or some pyrimidine (cytosine, uracil or thymine) [3,4]. These types of compounds have proved to be effective against HIV-1 and HIV-2, inducing cytopathogenicity in ATH-8 cells and inhibiting the expression of P-24 gag protein [5].

On the other hand, the synthesis of butenolides and saturated γ -lactones as an optically active fragment is currently receiving considerable attention in the light of their utility as synthons for the synthesis of biologically active natural products. Likewise, the γ -butyrolactone unit is one of the most widely present in bioactive natural products [6] and especially important are those functionalized γ -lactones in which the α position is either a methylene as in the antibiotic antitumor methylenoactocin [7] or dehydroprotolichestennic acids [8].

There have been important studies on stereoselective and non-stereoselective conjugated additions carried out on γ -lactones [9-12], although in comparison with other

acceptors, α,β -unsaturated sesquiterpene lactones, have received little attention.

To our knowledge not many amino acid sesquiterpene lactone Michael adducts have been reported to occur in a plant source [13-14] and in some cases insufficiently resolved ^1H nmr data and incomplete stereochemistry have been given.

Results and Discussion.

Recently it has been reported that the Michael reaction between the alantolactone and the dipeptide (Z)-L-Cys-L-Ala-OMe affords an adduct which showed interesting biological properties [15] where the biological role concerned with the activity of γ -lactone must take account the configuration of the new asymmetric carbon.

From these findings it seemed interesting to explore the 1,4-addition of 6-amino-1,3-dimethyl-2,4-pyrimidinedione **1** to the α,β -unsaturated system of the dehydrocostus lactone **2**, Ivalin acetate **3** and Zaluzanin A diacetate **4**. Compounds **2-4** are representative of well-known sesquiterpenes characterized by the presence of α,β -unsaturated γ -lactones. In this paper, we present a general approach to the synthesis of pyrimidine derivatives of an important class of bioactive compounds in which the type of compound to be obtained permits control of the absolute configuration in the final products.

Herein, we present the results obtained after addition of compound **1** to lactone **2** in methylene chloride solution in

the presence of Triton B (Method A, see Experimental) which afforded compound 5. In the proton nmr spectrum of compound 5 new signals at $\delta = 2.78$, and 2.72, were observed instead of the well identified signals of the C-13 exocyclic methylene ($\delta = 6.20$, d and 5.50, d from compound 2). In addition, also we were able to identify two singlets assigned for the N-Me groups at $\delta = 3.44$ and 3.32. This data suggests that the enolate of the substituted γ -butyrolactone was functionalized with the pyrimidine derivative 1. The Michael addition of compound 1 to the C-13 exocyclic methylene of compound 2 with the formation of a new C13-C5" bond was supported by the chemical shift of the C-13 methylene protons at $\delta = 2.78$, dd and 2.72, dd $J_{13-13'} = -15.0$.

The proton network of the new compound 5 was well established using the COSY spectrum. In this experiment we observed the correlations due H-6 with H-5 and H-7 as well those for H-11 with H-7, H-13 and H-13'. The H-11 signal in compound 5 appeared at $\delta = 2.37$ as a doublet of doublet of doublets $J_{7-11} = 12.0$, $J_{11-13} = 2.5$ and $J_{11-13'} = 6.0$. The large vicinal coupling observed between the H-7 and H-11 protons is indicative of an anti relationship between both protons. Using the well known Karplus approach, we have determined a dihedral angle between H-7 and H-11 at *c.a.* 160°. Consequently, because it is well known that H-7 possesses an α -configuration we

were able to assign unambiguously to H-11 a β -configuration. The full evidence about the stereochemistry of the stereocenter created was well determined by NOESY studies. From the NOESY spectra obtained from compound 5 and because it is well known that H-6 possesses a β -configuration, using the diagonal peaks from H-6 with the cross peaks from H-11 and H-8 β , we were able to prove the proposed configuration as is depicted in 5.

On the other hand, in the ^{13}C nmr spectra of compound 5, a new methylene signal at $\delta = 22.2$ was observed whose assignment was supported by the HMQC and HMBC experiments. The protons at $\delta = 2.78$ and 2.72 correlates with the C-13 carbon signal at $\delta = 22.2$. Likewise these protons correlate with C-12 ($\delta = 181.9$), C-11 ($\delta = 49.2$), C-7 ($\delta = 46.8$), C-4' (163.1), C-5' ($\delta = 85.8$) and C-6' ($\delta = 152.0$). Additional assignments are observed in Table 1.

The reaction of pyrimidine 1 with Ivalin acetate 3 using Method B gave the tetracyclic derivative 6 resulting from the 1,4-Michael addition of 1 through the C-5' carbon atom, followed by cleavage from the *Re* carbonyl face of the lactone ring by the C-6' amino group of the pyrimidine to provide stereoselectively compound 6. This compound shows in the ^1H nmr spectrum (DMSO- d_6) the absence of the characteristic acetoxy singlet suggesting that such groups suffered a hydrolysis. Furthermore, the H-8 signal is shifted to a higher field than that observed in compound 3. In order to promote solubility, compound 6 was acetylated using standard procedures, yielding compound 7. In derivative 7, two acetate signals were observed at $\delta = 2.03$ and 2.00. The C-11 proton signals at $\delta = 2.60$ (ddd, $J_{7-11} = 9.0$ Hz, $J_{11-13} = 6.5$ and $J_{11-13'} = 6.0$ and the value of $^3J_{7-11} = 9$ Hz measured by selective decoupling experiments, suggested that these two protons are in a *trans* configuration [15] The chemical shift of H-11 is ascribed to the deshielding effect arising from its position with regard to the carbonyl group. The axial proton of the methylene at C-6 is located at lower field ($\delta = 1.70$, ddd, $J_{5-6} = 12.5$, $J_{6-7} = 12.0$ and $J_{6-6'} = -12.5$). The deshielding effect compared with the same proton in compound 3 arises from its position, syn periplanar location with respect to the acetoxy group attached to C-8. The assignments of the carbon resonances of the products synthesized were carried out in deuteriochloroform by determining the HMQC and HMBC experiments. The long range HETCOR spectrum allowed us to distinguish among the five different carbonyl groups and between the five CH_2 groups as can be observed in Table 2.

The reaction of pyrimidine 1 with compound 4 proceeds either by method A or method B. In both cases the reaction gave in good yield stereoisomers 8 and 9 in a 1:1 ratio. In the structures of the derivatives 8 and 9, the Michael addition proceeds through C5' from the pyrimidine 1 to yield two isomers, C-13 α and C-13 β . Then,

Table 1

 ^1H and ^{13}C NMR Data of Compound 5 in Deuteriochloroform

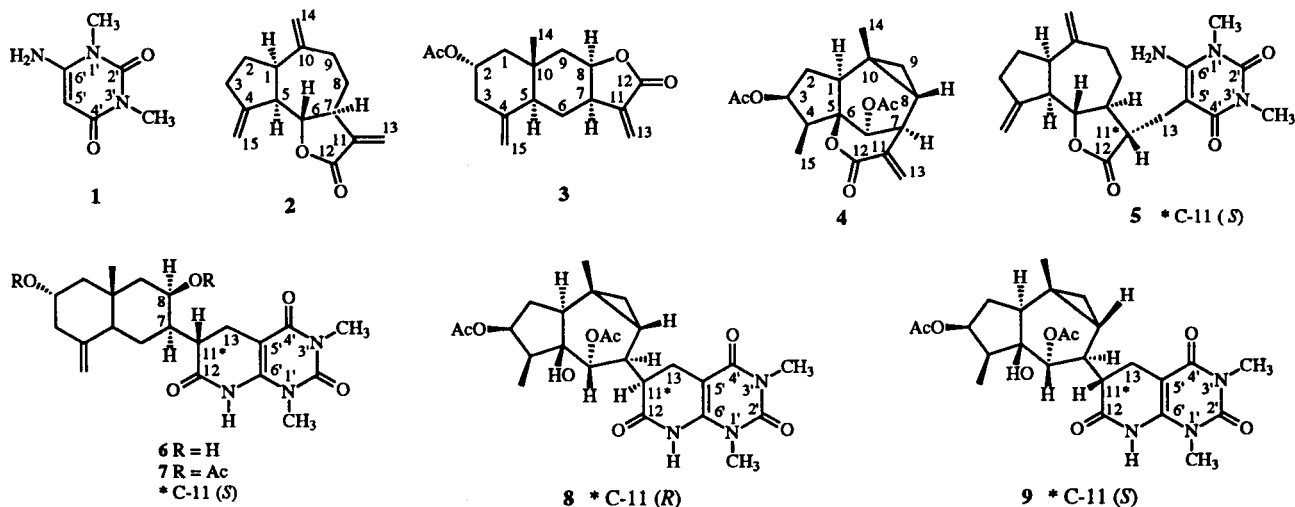
position	δH	multiplet	δC
1	2.87	m	47.3
2	1.94	m	30.1
	1.87	m	
3	2.54	m	32.5
	2.52	m	
4			151.9
5	2.80	br dd	51.7
6	3.98	dd	87.1
7	2.14	dddd	46.8
8	1.27	dddd	32.6
	2.68	dddd	
9	2.50	ddd	38.1
	2.05	ddd	
10			150.2
11	2.37	ddd	49.2
12			181.9
13	2.78	dd	22.2
	2.72	dd	
14	4.73	br d	111.5
	4.86	br d	
15	5.03	q	108.8
	5.13	q	
1' <i>N</i> Me	3.44	s	29.2
3' <i>N</i> -Me	3.32	s	28.2
2'			151.4
4'			163.1
5'			85.8
6'			152.0
NH ₂	5.62	br d	

Table 2
¹H and ¹³C NMR Data of Compounds 7, 8 and 9 in Deuteriochloroform

position	δ H	7 δ C	HMBC	δ H	8 δ C	HMBC	δ H	9 δ C	HMBC
1	1.20 dd 1.81 ddd	46.0	C ₁ C ₁₄ C ₂	1.33 dd	45.5	C ₂ C ₉ C ₁₀	1.65 dd	43.5 m	C ₉ C ₁₀ C ₂ C ₁₄
2	4.95 m	69.7		2.34 ddd 1.96 ddd	32.9	C ₅	2.47 ddd 1.95 ddd	33.0	C ₅
3	1.99 t 2.71 ddd	41.8	C ₅ C ₄ C ₂ C ₁₅	5.34 ddd	75.6		5.27 ddd	74.7	
4		145.7		2.06 dq	40.6	C ₁₅	1.87 dq	40.3	C ₁₅
5	1.85 d	49.3	C ₁₀ C ₁₄ C ₄ C ₁₀ C ₈	5.03 d	82.5			79.6	
6	1.56 br d 1.70 ddd	24.5			76.6	C ₅ C ₁ C ₇ C ₁₁	4.55 d	71.1	
7	1.92 m	38.9		1.55 dd	45.7		2.07	38.8	
8	5.15 m	68.9	C ₁₀	0.47 ddd	19.1		0.52 ddd	21.1	
9	1.36 dd 2.01 dd	43.4	C ₇ C ₁₄ C ₈	0.81 dd 0.13 dd	27.0	C ₁ C ₇	0.71 dd 0.16 dd	24.7	
10		35.0			15.5			15.5	
11	2.60 ddd	42.3		3.16 ddd	44.0	C ₁₃	3.05 ddd	46.0	C ₁₂
12		174.0			174.2			174.2	
13	2.70 dd 2.77 dd	19.9	C ₁₂ C _{5'}	3.02 dd 2.80 dd	22.7	C ₅ C ₆ C ₁₂ C ₅ C ₁₁	2.86 dd 2.98 dd	21.4	C ₁₁ C ₁₂ C ₇ C ₆
14	0.87 s	18.7	C ₅ C ₁ C ₉ C ₁₀	1.12 s	23.9		1.10 s	2.40	C ₉ C ₈ C ₁ C ₁₀
15	4.88 br d 4.58 br d	109.2	C ₅ C ₃ C ₅ C ₃	0.85 d	7.1	C ₅ C ₃ C ₄	0.80 d	6.5	C ₃ C ₄ C ₅ C ₁
HO-C ₅				3.63 d			2.59 d		
AcO-C ₃				2.10 s	171.1		2.09	171.0	
AcO-C ₆				2.01 s	21.2			21.1	
AcO-C ₂	2.03 s	170.0			174.4		1.93	170.0	
AcO-C ₈	2.00 s	21.3			21.2			21.1	
1'-NMe	3.44 s	170.4			21.3				
3'-N-Me	3.32 s	29.2	C ₂ C _{6'}	3.50	29.6	C ₂ C _{6'}	3.54	29.4	C ₆ C _{2'}
2'		28.2	C ₄ C _{2'}	3.37	28.3	C ₂ C _{4'}	3.38	28.3	C _{2'}
4'		151.4			151.0			151.2	
5'		163.1			161.4			161.9	
6'		85.8			89.8			88.2	
NH	9.1 br d	152.0	C _{5'}	8.4 br d	143.4		8.4 br d	143.7	

with the stereocenters created both structures suffered cleavage of the lactone with the amino group attached to C6' from the pyrimidine 1 with transformation into a lactam ring system leaving a hydroxy group attached to C-5 (see Scheme 1). The stereochemistry of the hydroxy group at C-5 could be established from the splitting pattern between C-4 α methine group and HO-C-5 ($^4J = 1.0$ Hz). This interesting W long range coupling was observed in both isomers 8 and 9 leaving consequently the C₅-OH group possessing a β -configuration. The C11 proton shows in both isomers a ddd signal, but the coupling constant between H7-H11 was very different; compound 8, $\delta_{H-11} = 3.16$, $J_{7-11} = 3.0$; compound 9, $\delta_{H11} = 3.05$, $J_{7-11} = 7.5$. The C-11 configuration in both isomers was achieved using the information afforded by NOESY experiments.

For compound 8, cross peaks for H-8 β /H-9 β ; H-8 β /H-14; H11 α /H-6; H-11 α /H-7 α H-6/H-15; H-9 α /H-1 α were noteworthy and they suggest that the H-7 and H-11 are oriented to the same side of the molecular plane. Based on the considerations mentioned we have assigned for C-11 of compound 8 the *R* configuration. For compound 9, (C-11 *S*) we observed in the NOESY experiment cross peaks between H-9 α /H-9 β ; H-9 α /H-1 α ; H-9 α /H-7 α , H-8 β /H-14; H-8 β /H-11 β ; H-8 β /H-13; H-8 β /H-13'. These observations supported the C-11 *S* configuration for compound 9. On the other hand, in compound 8, (C-11 *R*), the H-6 is deshielded to $\delta = 5.03$ (d, $J_{6-7} = 4.8$). The low field position of H-6 can be attributed to anisotropic effects from the lactam carbonyl group. In contrast, compound 9 (C-11 *S*) shows the H-6 proton at higher field ($\delta = 4.55$, d, $J_{6-7} = 2.0$).



The complete assignment of the ^1H and ^{13}C spectra of the compounds described in this paper was made by a variety of one and two dimensional nmr techniques, such as HOMO-COSY, TOCSY, NOESY, HMQC and HMBC (see Tables 1-2).

All the compounds, which were prepared, were screened for potential antitumor activity according to methods described earlier [16], but none of these compounds exhibited significant activity.

EXPERIMENTAL

The infrared spectra were recorded on a Nicolet FX-SX and Nicolet 55-X FT instruments. The mass spectra were recorded on a JEOL JMS-SX 10217 instrument. The 1D and 2D nmr spectra were recorded on a Varian Unity 300 and Varian Unity 500 spectrometers. All measurements were carried out using a 5 mm inverse detection probe. For the COSY experiment we used the standard Varian Unity program. All normal ^{13}C nmr spectra were acquired with composite-pulse decoupling, namely wideband alternating phase low-power technique for zero-residue splitting (WALTZ-16), as implemented in the spectrometer software. The HMQC experiment used the standard Varian Unity 500 pulse program optimized for short range couplings (140 Hz $^1\text{J}_{\text{C-H}}$). The sequence for the inverse-mode heteronuclear multiple bond correlation (HMBC) uses the Varian Unity 500 pulse program optimized for long range couplings (9 Hz $^3\text{J}_{\text{C-H}}$) and includes a low-pass filter to suppress one bond correlations (evolution time: 3.6 ms). The polarization transfer time was set to 90 ms to optimize for long-range heterocouplings. The recycling delays was 2.5 s. A total of 128 2K point spectra were acquired and zero-filled to 256 in t_1 . Window functions included sine bell shifted by $\pi/6$ in t_2 and 2-Hz Lorentzian broadening in t_1 dimension.

Method A.

To a solution of 775 mg of 1 (5 mmoles) and 1 ml of Triton B (40% aqueous solution) in 10 ml of a mixture THF-water

9:1, 230 mg of dehydrocostus lactone (2) in 5 ml of THF was added. The mixture was stirred for 3 hours at room temperature. The usual work up, followed by flash chromatography afforded compound 5, mp = 204-206°; ms: EI, m/z M+ 385 (12), 169 (55), 168 (100); ir: 3502, 3352, 3228, 1747, 1693, 1647, 1625, 1497 cm^{-1} .

Anal. Calcd. for $\text{C}_{21}\text{H}_{27}\text{N}_3\text{O}_4$: C, 65.44; H, 7.06; N, 10.90; O, 16.60. Found: C, 65.30; H, 7.01; N, 10.82; O, 16.87.

Method B.

To a solution of 290 mg of Ivalin acetate 3 (1 mmole) in 5 ml of methylene dichloride, 310 mg of 1 (2 mmoles), 0.5 ml of 2.5 M aqueous sodium hydroxide and 50 mg of Aliquat 336 were added. The reaction was stirred at room temperature for 24 hours. After the usual work up, the residue was purified by flash column chromatography (silica gel) to yield 6, mp = 194-195°; ms: EI m/z M+ 403 (20), 168 (100), 207 (10), 155 (25); ir: 3458, 3271, 2917, 1696, 1646, 1515, 1217 cm^{-1} ; $\text{C}_{21}\text{H}_{28}\text{O}_5\text{N}_3$, MW = 403.

Compound 6 was immediately acetylated under standard conditions to yield 7, mp = 254-255°; ms: FAB, m/z M+ 488 (100), 428 (12), 368 (50), 208 (65); ir: 3411, 2954, 2929, 2856, 1732, 1701, 1654, 1365 cm^{-1} .

Anal. Calcd. for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_7$: C, 61.59; H, 6.82; N, 8.62; O, 22.97. Found: C, 61.50; H, 6.90; N, 8.60; O, 23.0.

Compounds 8 and 9 were prepared using method A or method B and after the usual work up, they were purified by direct separation by using hplc on a Varian 5000 instrument with a uv detector (210 nm) using a reversed phase column Sufelcosil LC-18 semipreparative (25 cm x 10 mm ID), with a solvent system water: acetonitrile 60:40, flow 2.5 ml/minute.

Compound 8 had mp = 152-153°; ms: EI m/z M+ 503 (5) 208 (100); ir: 3993, 3411, 2954, 2929, 2856, 1732, 1701, 1654, 1510 cm^{-1} .

Anal. Calcd. for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_8$: C, 59.63; H, 6.61; N, 8.34; O, 25.42. Found: C, 59.59; H, 6.65; N, 8.28; O, 25.48

Compound 9 had mp = 146-147°; ms: EI, m/z M+ 503 (5) 384 (5), 208 (100); ir: 3691, 3413, 2956, 1730, 1699, 1656, 1510 cm^{-1} .

Anal. Calcd. for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_5$: C, 59.63; H, 6.61; N, 8.34; O, 25.42. Found: C, 59.68; H, 6.55; N, 8.27; O, 25.50.

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