REMARKABLE REGIOSELECTIVE ENZYME - MEDIATED ACYLATION OF GINSENOSIDES Rg_1 and Rb_1

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In traditional Chinese medicine, the roots of *Panax ginseng* C.A. Meyer(Araliaceae) have been used in both processed and unprocessed forms such as Ginseng Radix Rubra (red ginseng) and Ginseng Radix(white ginseng). The pharmacologically active constituents of these ginsengs(the ginsenosides) have been deeply pursued and, after extensive chemical studies corroborated by selective chemical and enzymatic hydrolysis, furthermore characterized as complex dammarane - type triterpene oligoglycosides(1).

Recently, a closer examination of the water soluble portion of white ginseng, compared with red ginseng(2), revealed the presence of considerable amounts of four malonylated ginsenosides, namely malonyl ginsenosides Rb_1, Rb_2, Rc and Rd together with previously known ginsenosides. By a combination of chemical and spectroscopic evidences, including the ^{13}C - NMR and the metastable ion analysis of liquid secondary ion mass spectra(SIMS MS - MS), the four malonyl - ginsenosides have shown to contain the malonyl residue attached to the primary hydroxyl function of the terminal glucosyl moiety. It is important to highlight that these compounds behave as acidic saponins due to the half - ester forms of their malonyl residue and not only are they more soluble in water as the ordinary ginsenosides, but they also cause a remarkable increase of the water solubility of the other ginsenosides.

In spite of these interesting properties which can deeply influence the absorption of these drugs in humans, to the best of our knowledge no information is available on the synthesis of malonyl ginsenosides and in general of specific esters of ginsenosides with aliphatic carboxylic acids.

The regioselective acylation of glycosides still represents a challenging problem due to the multiple hydroxyls present in the molecule.(3) Although primary OH's are normally the most reactive towards the acylation reaction, a clear discrimination between primary and secondary hydroxyls usually involves multistep protection and deprotection procedures. Furthermore, the distinction among primary OH's of various saccharide moieties remains a difficult task.(4)

In the last few years we have successfully exploited the ability of the proteolytic enzyme subtilisin(protease Carlsberg) to catalyze the transesterification reaction of polyhydroxylated compounds and activated esters in anhydrous polar organic solvents.(5) As an example, different flavonoid monoglucosides or disaccharide monoglycosides have been regioselectively butanoylated at the primary OH of glucose. Only in particular cases, esterification occurred at secondary OH's.(5c)

This methodology seemed to us very attractive for the regioselective esterification of other more complex glycosides and to broaden its application we have examined the behaviour of various ginsenosides. The aim was to obtain new derivatives having modified solubility properties with respect to the ordinary ginsenosides and, eventualy, to have a synthetic access to the malonyl - ginsenosides.

We started quite recently this research program, and we wish to report here the conclusive results obtained with the structurally simple 6,20 - di - O - glucopyranosyl - 20(S) - protopanaxatriol ginsenoside Rg_1(Rg_1) 1 and some preliminary data on the more complex ginsenoside Rb_1(Rb_1)2.

RESULTS

Following the protocol for the esterification of flavonoid glycosides(5a, c), Rg_1 was incubated with an excess of 2, 2, 2 - trifluoroethylbutanoate and in the presence of subtilisin Carlsberg in pyridine at 37 ℃. After stirring for 24 h, TLC analysis revealed an almost 30% conversion to a mixture consisting of at least three main components with no selectivity.(6)

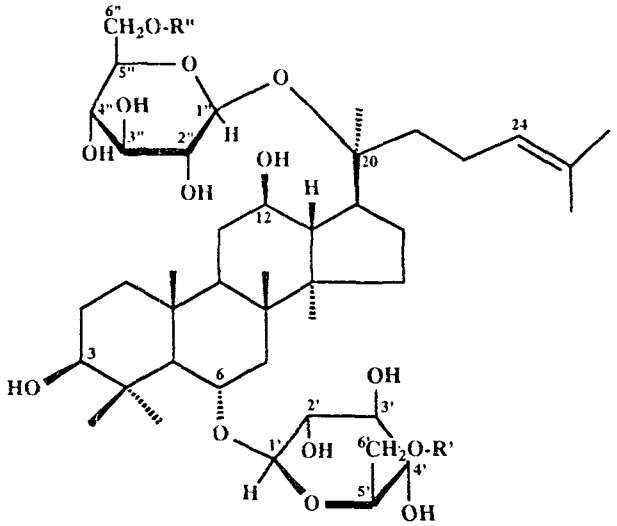
In order to overcome these unsatisfactory results we looked for more selective biocatalysts and we turned our attention to the lipases, a group of enzymes known to catalyze the enantioselective esterification of alcohols.(7)

However, lipases are not active at all or scarcely active in pyridine and in other polar solvents like DMSO and DMF. Therefore other suitable reaction media had to be found. After many trials, the most satisfactory results with respect both to the activity of the enzyme and to the solubility of substrate were obtained in *t* - AmOH.

Various commercially available lipases were tested, lipase from *Chromobacterium viscosum, Mucor mijehi, Pseudomonas ceapacea, Candida antarctica* and pig pancreas. 2,2,2, - Trifluoroethylbutyrate and vinylacetate were employed as acylating agent with similar results. The latter reagent was thoroughly used in this project

because of the easier interpretation of spectroscopic data.

The best selectivity was displayed by *Candida antarctica* lipase(Novo) supported on a macroporous acrylic resin, it gave a neat conversion to only two products. In a typical experiment, 100mg of Rg_1 in 3.5mℓ *t*-AmOH were treated with 3.5mℓ of vinylacetate and 0.5g of the supported enzyme. The suspension was stirred for 24 h at r.t. until the starting material disappeared. The enzyme was filtered, the solvent evaporated and the two products isolated by FC with the solvent system AcOEt/MeOH/H_2O 90 : 10 : 5. 10mg of the less polar products(Rf 0.36, AcOET/MeOH H_2O 100 : 20 : 5 ; Rg_1 has Rf 0.19) and 42mg of the second product(Rf 0.29) were obtained and characterized as follows : 6',6"-di-O-acetyl-ginsenoside Rg_1 3 and 6'-O-acetyl-ginsenoside Rg_1 4.



1 R' = R" = H ginsenoside Rg1
3 R' = R" = Ac
4 R' = Ac; R" = H
5 R' = $OCOCH_2COOCH_3$, R" = H
6 R" = $OCOCH_2COOCH_2CF_3$, R" = H

The positive FAB-MS spectrum(8) of the less polar compound showed an intense $(M+Na)^+$ peak at m/z 902 and a weak $(M+H)^+$ at m/z 885, in accordance with the presence of two acetyl group in the molecule, together with numerous fragment ion peaks. In addition to the loss of 42 and 60 amu fragments, the pseudomolecular ions showed two consecutive losses(B E) of acetylated glucose, to afford for the protonated molecule daughter ions at m/z 663 and 441. The two acetyl groups are thus each attached to a glucose moiety and the position of attack was established by inspection of the ^1H-NMR spectrum at 300 MHz(9).

The spectrum displayed two CH_3COO singlets at 1.99 and 2.00ppm and low-field resonances easily attributable to the the following protons on the basis of chemical shift analogies and multiplicity :

H-24	5.08 ppm	t	J=7 Hz
H-6ax	3.92	td	11, 11, 3.5
H-12ax	3.54	td	11, 11, 5.5
H-3ax	2.92	dd	11.5, 5.5

The anomeric protons resonated at 4.49 and 4.35 ppm both as d, J=7.8Hz. The other oxymethine protons gave rise to a complex signal in the 3-3.4 ppm zone. Only the oxymethylene protons were recognized as two AB portions of ABX systems at 4.37 and 4.31ppm(J=11.5, 2 Hz, A parts) and 4.01 and 3.96 ppm(J=115, 7 Hz, B parts). In Rg_1, the last mentioned signals occurred overlapped at 3.68 and 3.68 ppm. The less polar product is therefore 6',6"-di-O-acetylginsenoside Rg_1 3.

The second reaction product contained only one acetyl group, as suggested by $(M+Na)^+$ and $(M+H)^+$ peaks at m/z 865 (base peak) and 843, and by $(M-H)^-$ at m/z 841 (base peak), presumably located at one of the primary OH's.

To have an idea, we examined the B/E linked scan spectra. In the negative mode, the $(M-H)^-$ peak showed the gas-phase unimolecular decomposition to either ions at m/z 679 and 637 which was due to the loss of glu and Acglu through cleavage of glucosidic bond accompanied by the transfer of hydrogen from the leaving sugar. At variance, the weak $(M+H)^+$ ion showed the loss of glu and glu+H_2O generating ions at m/z 663 and 645 resp. The ion at m/z 645 was then selected as a parent and a fragmentation was observed to obtain the m/z 441, 423(intense) and 405 ions. According to data found in literature(1, 2, 10, 11), these discoveries led to the suggestion that acetylation occurred at glucose linked to the C-6OH.

In a first attempt to verify this assignment, the monoacetylated Rg_1 was subjected to an enzymatic hydrolysis with crude hesperidinase. This enzymatic preparation is reported to split off the glucose moiety at C-6OH of Rg_1 to give the 20-O-glucopyranosyl-20(S)-protopanaxatriol[Ginsenoside F_1, (F_1)] (5), albeit in very low yield(10).

300mg of monoacetyl-Rg_1 in 50mℓ 0.2M phosphate buffer pH 3.8 were incubated with 8g hesperidinase(Sigma, 0.01U/mg) at 40℃ for 2 days. After usual work-up, 180mg of F_1 was obtained, identified by comparing the ^{13}C-spectrum with the reported one(12).

Unfortunately this result could not be kept as proof of the acetylation site because, by monitoring the reaction step-by-step, we could observe the initial loss of the acetyl group to give Rg_1, followed by the hydrolysis of the glucose moiety at C-6OH.

For an unequivocal structural proof of monoacetyl-Rg_1, a thorough NMR study was then undertaken.

In spite of the pharmacological importance of ginsenosides, only ^{13}C-NMR data are available(1, 12, 13,14). C-1' and C-1" were assigned for Rg_1, but due to close proximity ambiguities in the spectral analysis of the genine and in particular of the attribution of the resonances of C-3, C-4,

C-5 and C-6 of glucose moieties remained. This prevented the use of ^{13}C-NMR spectroscopy and in fact we could only establish the acetylation of a primary OH through observation of a downfield triplet at 64.4 ppm with respect to 61.46 ppm for the unsubstituted oxymethylene group.

As regards the ^{1}H-NMR, the extensive signal overlapping of aliphatic and sugar protons discouraged the use of medium field instruments and of traditional techniques for assignment. Few data are currently reported in literature, relevant to H-24, anomeric protons(not assigned) and methyls(1, 2).

We devised a strategy to determine the esterification site based on the correlation of the acetylated oxymethylene signal with the resonance of the corresponding anomeric proton using the vicinal $^{1}H-^{1}H$ coupling interactions. The signals of the anomeric protons could then be assigned to the C-6OH glucose(glucose') or to the C-20OH glucose(glucose") *via* one-bond $^{13}C-^{1}H$ couplings, substantiated by pertinent NOE connectivities.

This strategy could be realized thanks to the intrinseca lly high resolving power of a 600 MHz instrument (9) wh ich permitted a better distribution of ^{1}H chemical shifts of the sugars hydrogens and allowed a complete spectral analysis by the concomitant use of modern pulse sequences. Based on these physicochemical evidence, the structure of the monoacetylated Rg_1 has been elucidated and confirmed to be 6'-O-acetyl-ginsenoside Rg_1 4.

The ^{1}H-NMR spectrum is reported in Figure 1. The NMR data are collected in TABLE 1, and were determined by performing the following experiments.

-A homonuclear COSY in the DQF mode could localize a few connections :

H-3 to H-2ax and H-2eq,
H-6 to H-5, H-7ax and H-7eq,
H-12 to H-11 and H-13
H-24 to H-23a, H-23b, CH_3-25 and CH_3-26.

In the sugar units, the two anomeric protons at 4.42 and 4.29 ppm were linked to the adjacent protons resonating at 2.87 and 2.98 ppm respectively(Figure 2). These in turn were correlated to the same triplet signal at 3.18 ppm due to the fortuitous overlapping of the two C-3 protons for both glucose units. Also the C-4 protons were almost superimposed at 3.01-3.02 ppm.

-A total correlation spectroscopy(TOCSY) experiment was therefore performed to find a correlation between protons that belong to the individual monosaccharide units. This allowed to recognize that the low-field anomeric proton was related to the H_2-6 signals at 3.62 and 3.40, and that the high-field anomeric proton was located on the same glucose moiety as H_2-6 at 4.32 and 3.91 ppm(Figure 3). As regards the tetracyclic skeleton, this experiment enabled to find, among other signals, the structurally relevant H-17 at 2.18 ppm(see below).

-^{1}H broad band decoupled ^{13}C-NMR spectrum to measure chemical shifts followed by an APT experiment to assign multiplicity. On the basis of comparison made on literature data of Rg_1, F_1 and the 6-O-monoglucosyl-20(*S*)-protopanaxatriol Rh_1(1, 12), C-1' was found at 104.45 and C-1" at 96.55ppm.

-$^{13}C-^{1}H$ COSY *via* one-bond couplings which allowed the assignment of all carbons directly bound to the protons already revealed by the above experiments. Taking advantage of the complete chemical shift resolution in the ^{13}C frequency domain, several unassigned proton signals were attributed.

In this way, the anomeric protons at 4.29 and at 4.42 ppm were assigned respectively to H-1' and to H-1", therefore the structure of 6'-O-acetylginsenoside Rg_1 4 has been completely substantiated.

To further support this assignment, an analysis of the through space connectivities was undertaken with the help of a two dimensional nuclear Overhauser effect spectroscopy(NOESY) experiment. Crucial to this point was the observation that C-12OH(easily recognized at 4.59 ppm by its coupling to H-12) and H-17 had, among others, a strong interaction with H-2" and H-1" respectively(Figure 4).

These interactions were useful to define the mutual orientation of C-20 OH glucose with respect to the tetracyclic skeleton. The NOE contacts were used to drive the energy minimization of a molecular model, by means of restrained molecular mechanics calculations(15). The resulting conformation, depicted in Figure 5, is in complete agreement with the experimental data.

The next step of our research was the introduction of a malonyl unit into the Rg_1 molecule. Previously we have shown that methyl trifluoroethylmalonate is a suitable reagent for the regioselective malonylation of the flavonoid glucoside isoquercitrine(16). The activated trifluoroethylcarboxylate group formed an acyl enzyme intermediate which was attacked by the nucleophilic 6-OH of the glucose moiety to develop the corresponding methyl malonyl isoquercitrine.

When Rg_1 was incubated under the above described conditions in the presence of methyl trifluoroethylmalonate, the starting material completely disappeared after 24h, and a main product was obtained together with a small amount of a less polar component(Rf 0.24 and 0.30 resp., AcOET/MeOH/H_2O 80 : 15 : 5 ; Rg_1 has Rf 0.15).

The main product was characterized as 6'-O-methyl malonyl-Rg_1 5 on the basis of its spectroscopic properties in comparison with those of 4.

The positive FAB-MS spectrum(8) displayed a $(M+H)^+$ ion at m/z 901, and the metastable ions analysis (B/E) showed the preminent loss of 162 and 180 amu due to the elimination of the C-20OH glucose.

The ^{1}H-NMR spectrum at 300MHz(9) was similar to that of 4, with almost the same resonance positions of the

Table 1. NMR data of 6' - O - acetylginsenoside Rg_1 4.

protons	δ (*ppm*)	*mult*	*J*(*Hz*)	*NOE to protons* *	*carbons*	δ (*ppm*)
H - 1ax	0.93	m			1	38.86
H - 1eq	1.58	dt	13, 3, 3		2	26.70
H - 2ax, eq	1.4 - 1.5	m			3	76.83
H - 3ax	2.92	dt	11.5, 5.5, 5.5	H - 1ax, H - 5ax, CH_3 - 28	4	40.10
H - 5ax	0.97	d	11		5	60.54
H - 6ax	3.89	td	11, 11, 3.5	H - 7eq, CH_3 - 18, 19, 29, H - 1'	6	77.84
H - 7ax	1.43	m			7	44.52
H - 7eq	1.88	dd	13, 3.5		8	41.00
H - 9ax	1.30	dd	13, 3		9	49.03
H - 11ax	1.01	m			10	39.50
H - 11eq	1.63	m			11	30.60
H - 12ax	3.54	tdd	11, 11, 5.5, 1.5	H - 17, H - 9, CH_3 - 30, OH - 12	12	69.38
H - 13ax	1.53	t	11, 11		13	48.43
H - 15ax	0.91	m			14	51.30
H - 15eq	1.43	m			15	30.60
H - 16ax	1.2	m			16	26.05
H - 16eq	1.78	m				
H - 17ax	2.18	td	11, 11, 9	H - 1", OH - 12, H - 12, H - 16a, b	b1	50.59
H - 22a	1.44	m			22	35.65
H - 22b	1.71	m				
H - 23a	1.93	m			23	22.61
H - 23b	2.05	m			24	126.07
H - 24	5.07	bt	7		25	130.80
CH_3 - 18	0.96	s			18	17.01
CH_3 - 19	0.87	s			19	17.30
CH_3 - 21	1.245	s		H - 1"	21	21.70
CH_3 - 26	1.56	bs			26	17.87
CH_3 - 27	1.64	bs			27	30.58
CH_3 - 28	1.235	s		H - 3	28	30.58
CH_3 - 29	0.86	s		H - 6ax	29	15.77
CH_3 - 30	0.825	s		H - 12, H - 17	30	17.11
H - 1'	4.295	d	7.8	H - 3', H - 5', H - 6ax	1	104.45
H - 2'	2.985	ddd	9, 7.8, 5.4		2'	73.43
H - 3'	3.18	t	9, 9		3'	76.72
H - 4'	3.02	dd	9, 8.5		4'	69.53
H - 5'	3.325	ddd	8.5, 7.2		5	73.13
H - 6'a	4.32	dd	11.5, 2.0		6'	64.40
H - 6'b	3.91	dd	11.5, 7			
H - 1"	4.42	d	7.8	H - 17, H - 3", H - 5", CH_3 - 21	1"	96.55
H - 2"	2.87	td	9, 7.8, 4.5	OH - 12, H - 4"	2"	73.29
H - 3"	3.18	t	9		3"	76.72
H - 4"	3.03	dd	10, 9		4"	69.53
H - 5"	3.065	ddd	10, 6, 2		5"	76.15
H - 6"a	3.625	ddd	11.5, 6, 2		6"	61.46
H - b"b	3.40	ddd	11.5, 6, 6			

oH - 3 4.13, OH - 12 4.58, OH - 2' 4.69, OH - 3' - -, OH - 4' 5.07, OH - 2" 4.77, OH - 3" - -, OH - 4" 5.82, OH - 6" 4.20.

* only structurally relevant connectivities are reported(strong interactions underlined).

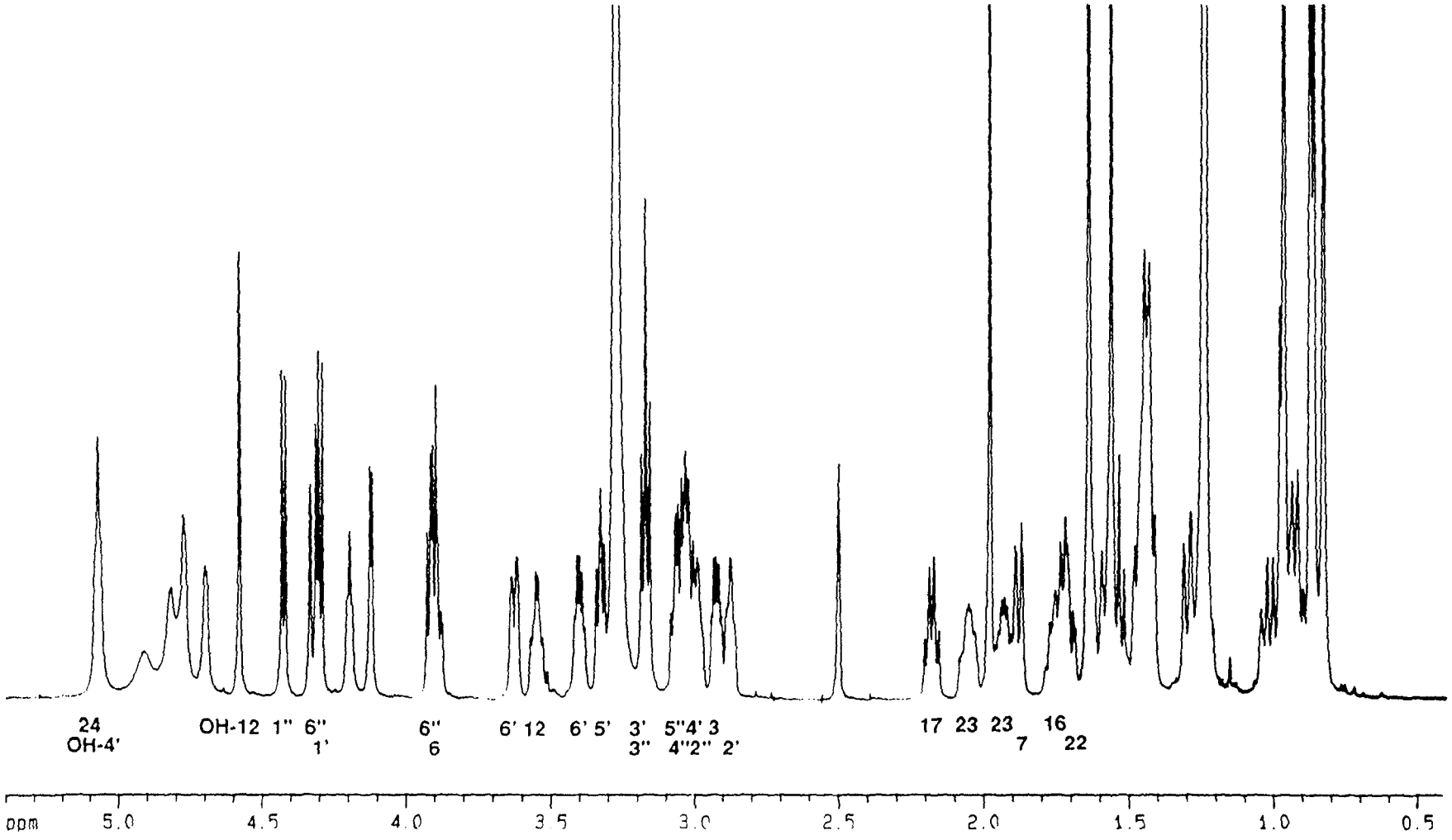


Figure. 1. ^{1}H - NMR spectrum of 4(600MHz, DMSO - d_6 35℃).
Numbering below signals refers to protons.

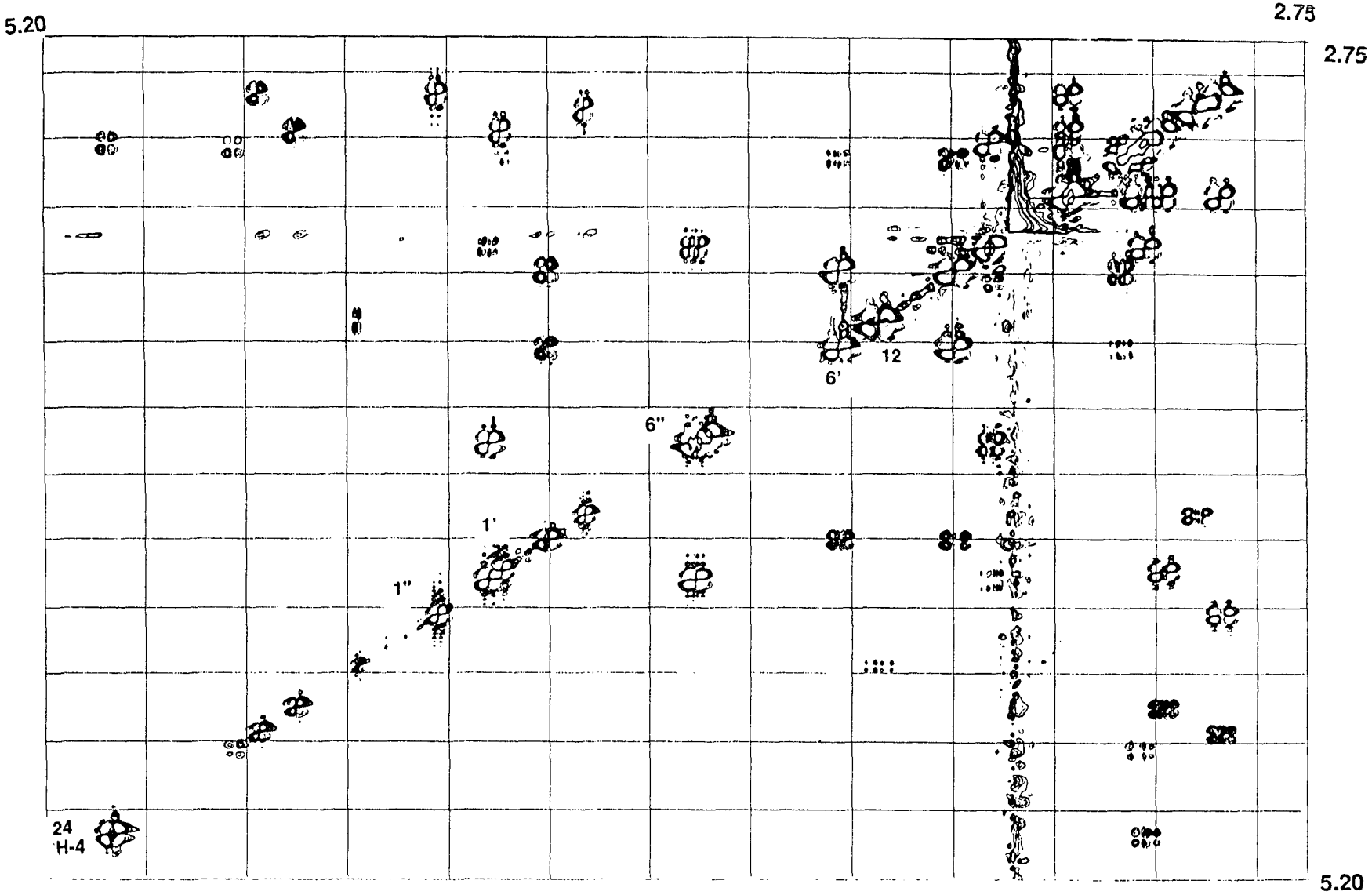


Figure 2. Subsection(2.75 - 5.20 ppm) of the $^1H - ^1H$ COSY spectrum of 4.
Numbering of the contour plot maps refers to protons.

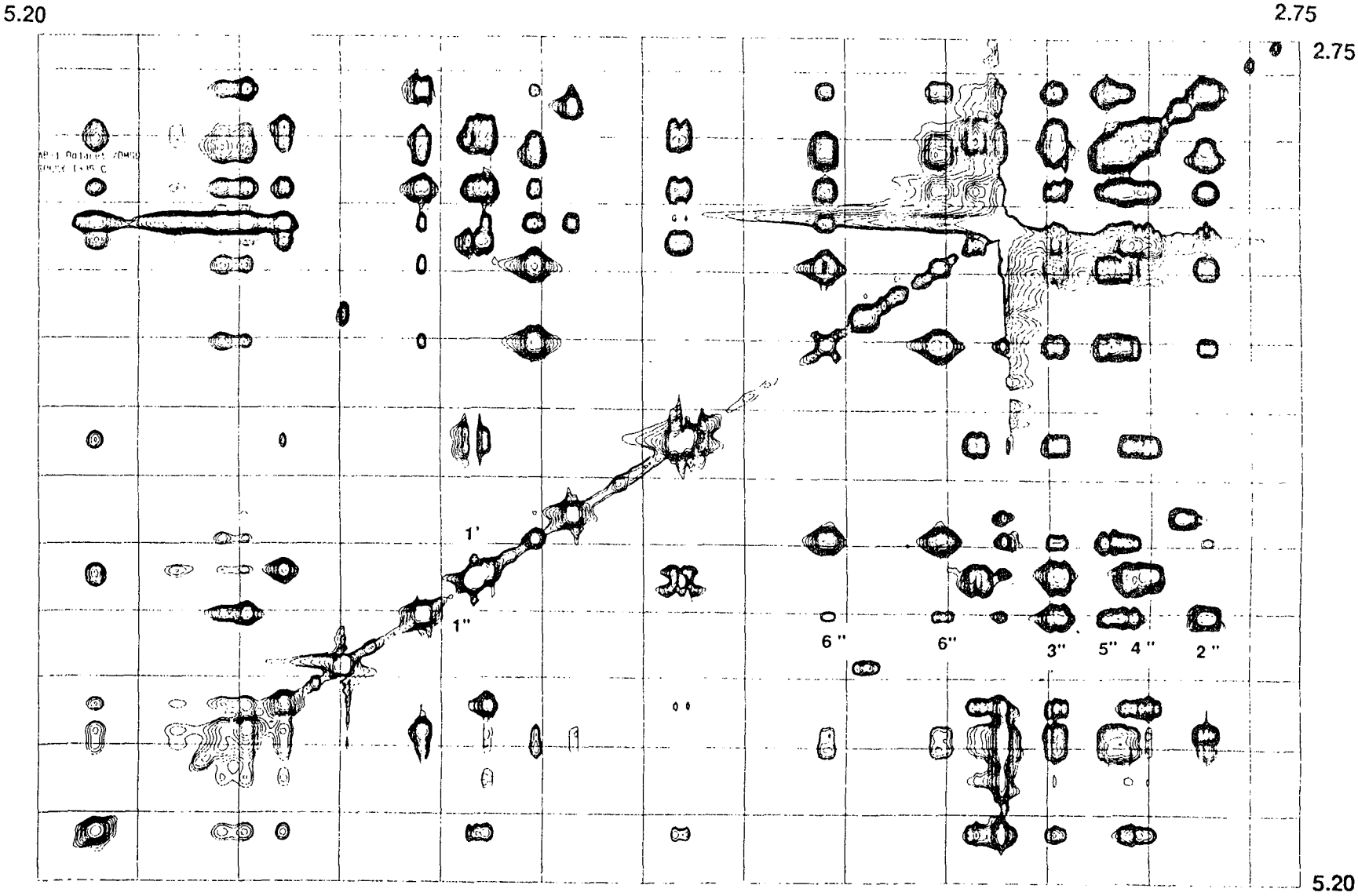


Figure 3. Subsection(2.75 - 5.20 ppm) of the TOCSY spectrum of 4.

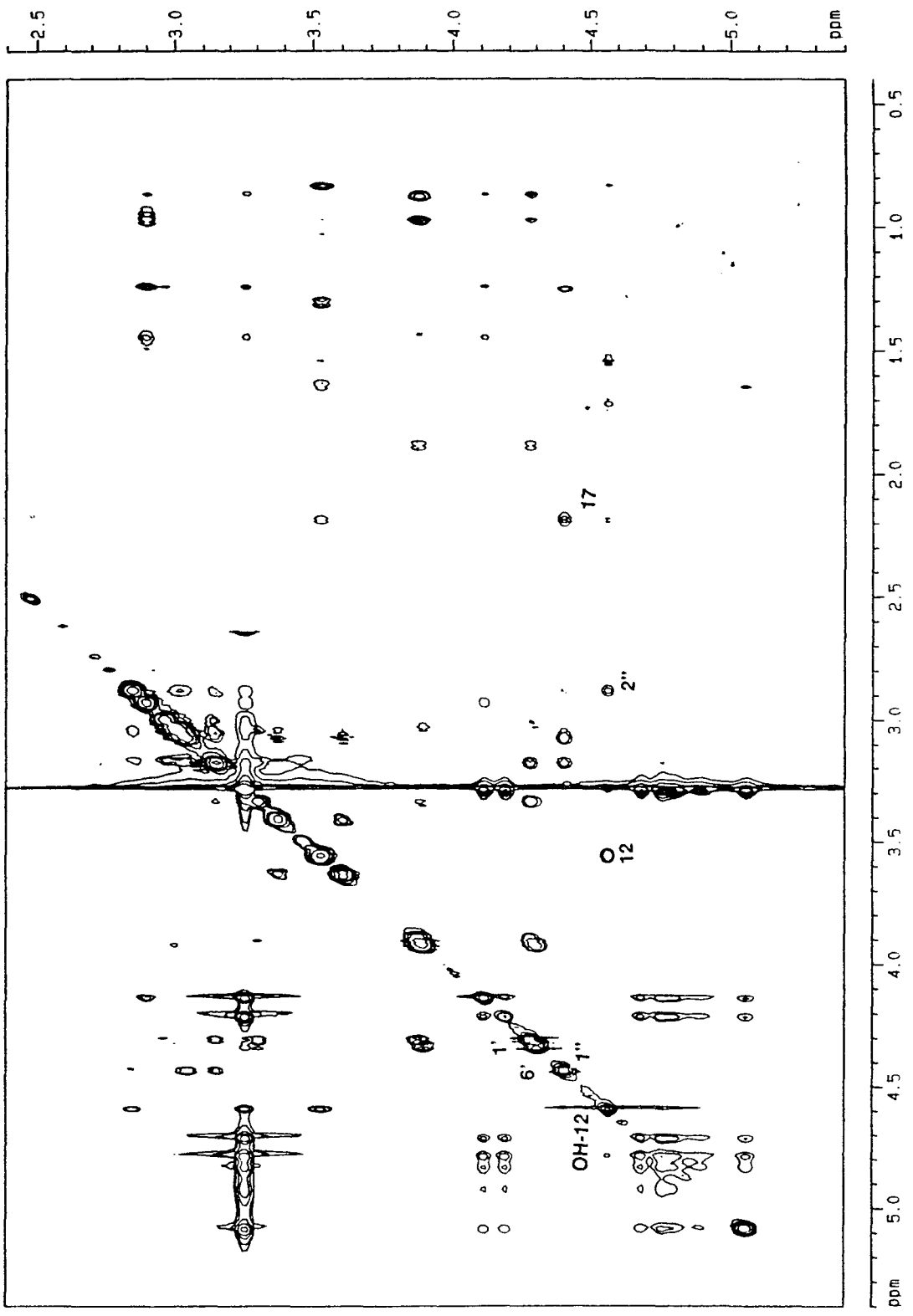


Figure 4. Subsection of the NOESY spectrum of 4.

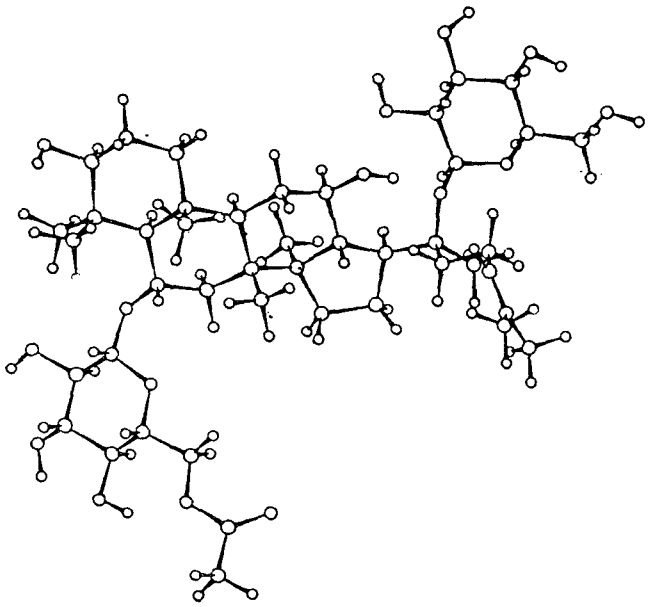


Figure 5. Minimum energy conformation of 4.

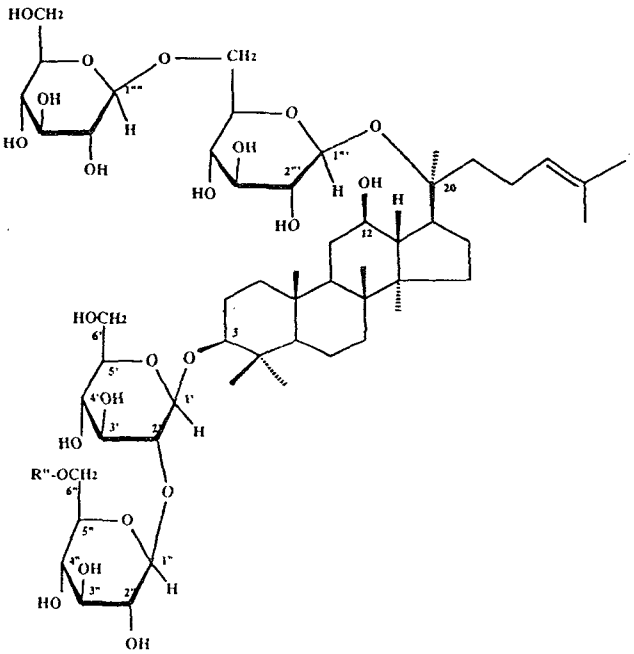
anomeric protons(H-1' and H-1" at 4.35 and 4.48 ppm), of the esterified oxymethylene protons(H-6'a and H-6'b at 4.46 and 4.03 ppm), of the oxymethine protons(H-6"a and H-6"b at 3.66 and 3.47 ppm), and of the oxymethine protons(H-5' at 3.38, H-3' and H-3" overlapped at 3.24, H-2" at 2.94 ppm). In addition, a correlation was observed among H-1", H-2", H-6"a and H-6"b(TOCSY). The presence of the methyl malonate moiety was inferred from a singlet at 3.67 ppm(5H) due to the superimposition of methylene and methoxycarbonyl signals, and from the ^{13}C resonances at 167.0, 166.6(esters CO's), 52.2($COOCH_3$) and 41.4 ppm($OCO-CH_2-COO$).

The less polar component was identified as 6'-O-trifluoroethyl malonyl-ginsenoside Rg_1 6 by the FAB MS spectrum[$(M+H)^+$ at m/z 969] and by the ^1H-NMR spect rum which contained a $-CH_2-CF_3$ quartet at 4.77 ppm, J= 8.5Hz. The other structurally useful proton signals were similar to those of 4 and 5.

The presence of this by-product is quite surprising because of the overwhelming propensity of the trifluoroethyl ester group to form the acyl enzyme intermediate with respect to the carbomethoxy function(trifluoroethanol is a better leaving group than methanol).

Work is in progress to try to hydrolyze selectively the $COOCH_3$ function in the presence of COOGlu function in order to obtain the 6'-O-malonyl ginsenoside Rg_1. Chemical means, e.g. excess LiBr in DMF containing 1eq of AcONa as methyl scavenger at 100℃, appeared too harsh and non-selective giving a complex mixture of products among which only Rg_1 could be identified. We hope to achieve our goal by using milder conditions and to this purpose we are currently exploiting the use of esterases, which are well known enzymes widely employed in the enantioselective and regioselective hydrolysis of methyl esters(7). We already used this biomethod to hydrolyze selectively the $COOCH_3$ group of the above me ntioned methyl malonylisoquercitrine to malonylisoquercitrine in acceptable yield(16).

Encouraged by the promising results obtained with Rg_1, we faced a more challanging problem to acetylate the disaccharide diglucoside ginsenoside Rb_1(Rb_1) 2, with the hope that the enzyme would be able to discriminate among the four glucose moieties and various primary and secondary OH's. Moreover, Rb_1 occurs in nature as a malonylated derivative, having the malonyl residue at the primary 6"-OH (2).



2 R" = H ginsenoside Rb1
7 R" = Ac

Candida antarctica lipase worked very efficiently with Rb_1 under the same conditions as for 1. Two products were obtained : a small amount of a diacetylderivative[Rf 0.31, AcOEt-MeOH-H_2O 9 : 4 : 1, Rb_1 has Rf 0.14. $(M+Na)^+$ and $(M+K)^+$ ions at m/z 1215 and 1231 in FAB+MS] and a larger amount of a monoacetylated compound[Rf 0.23, $(M+Na)^+$ and $(M+K)^+$ at m z 1173 and 1189].

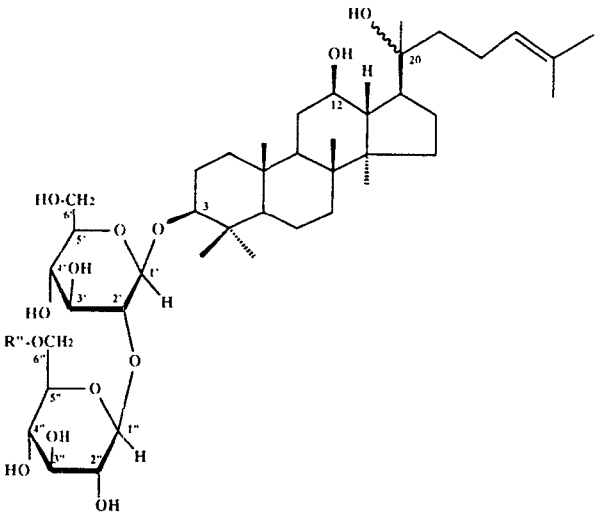
The structural analysis of the two reaction products was a very difficult task even using the 600 MHz NMR instrument. At present we have had only preliminary data and an indirect evidence which suggested that the monoacetylderivative had structure 7, with the acetyl group attached at the terminal glucose of the glucopyranosyl (1→2)-glucopyranosyl moiety at C-3OH.

As for the MS fragmentation of Rb_1 and its 6" - O - methylmalonylderivative(2), the B E linked scan spectrum of cationated molecular ions at m z 1173 and 1189 showed a loss of glu - O - glu and glu - O - gluOH units attached at C - 20OH which generated ions at m z 849, 831 and 865, 847 respectively. In addition, the abundant source ion at m z 831 was a precursor of a fragment at m z 627, due to the loss of a terminal Acglu. In the 600MHz NMR spectrum(9) of 7 we could observe four doublets of the anomeric protons at 4.52, 4.47, 4.32 and 4.28 ppm(all d, J=7.8Hz), two downfield signals at 4.31(dd, J=11.5, 1.5Hz), and 4.07 ppm(dd, J=11.5, 6.5Hz) of an acetylated CH_2OH, and a signal at 3.91 ppm(dd, J=11.5, 1.5Hz) due to one proton of the CH_2-O bridge in the glucopyranosyl(1·6)-glucopyranosyl moiety. The other oxymethylene and oxymethine protons, together with H-3 and H-12, were obscured by overlapps which gave rise to a complex, not yet analyzed envelope between 2.9 and 3.7 ppm.

Furthermore, the triplet at 63.80 ppm in the ^{13}C-NMR spectrum substantiated the acetylation of a CH_2OH, but the coincidence of the resonance of the three CH_2OH in Rb_1 at 61.40 ppm prevented a distinction.

To strenghten the structural proposal and, more specifically, with the aim to gain information on the chemical shift of the glucose protons spin systems, we decided to hydrolyze Rb_1 with AcOH 40% obtain the disaccharide monoglucoside ginsenoside Rg_3(Rg_3) 8, to study its acetylation and to investigate in detail the spectroscopic characteristics of the reaction product. These data will be utilized in the future for a decisive interpretation of the proton spectrum of 7.

Rg_3 was obtained as an inseparable mixture of 20(S)-20(R)-epimers(1), as indicated by the apparent quartet of H-24 at 5.08 ppm, due to the superimposition of two close triplet(J=7Hz), by the splitted singlet of CH_3-27 at 1.61 and 1.62 ppm and by the two C-24 signals at 125.30 and 125.26 ppm.



8 R" = H 20(R,S)-ginsenoside Rg3
9 R" = Ac

Enzymatic acetylation of Rg_3 with the catalysis of *Candida antarctica* lipase in THF, proceeded smoothly developping in almost quantitative yield the 6" - O - acetyl - ginsenoside Rg_3 9[(M - H) at m z 825, gas - phase loss of 204 amu to form an intense m z 621 ion] with the same initial reaction rate as for 2.

The ^1H-NMR spectrum contained two doublets of the anomeric protons H-1" and H-1' at 4.46 and 4.27 ppm (J=7.8Hz). By ^1H-^1H-COSY and TOCSY experiments, the former signal was correlated to low-field H_2-6" at 4.17 (dd, J=11.5, 1.5Hz) and 3.97 ppm(dd, J=11.5, 6Hz) through H-2" at 3.02(t, J=7Hz), H-3" at 3.18(t, J=7Hz), H-4" at 3.09(m) and H-5" at 3.30 ppm(ddd, J=7.5, 6, 1.5Hz). The second anomeric proton was part of a spin system made by H-2' at 3.26(t, J=7Hz, at low field due to the glucosidic linkage), H-3' at 3.38(t, J=7Hz), H-4' and H-5', at 3.09(m), H_2-6' at 3.63(dd, J=11.5, 1.5Hz) and 3.41 ppm(dd, J=11.5, 5Hz). H-1" showed NOE contacts to H-2' and H-1' (inter alia), whereas H-1' had a structurally relevant connectivity to H-3 at 3.01 ppm.

The similar behaviour of Rb_1 and Rg_3 towards enzymatic acylation suggests an approach to the enzyme active site by the two compounds with the identical position of their molecule, again supporting the acetylation of the 6" - OH of the ginsenoside Rb_1.

CONCLUSION

It has been found that the enzyme *Candida antarctica* lipase promotes efficiently the regioselective esterification of ginsenoside Rg_1 1 and ginsenoside Rb_1 2 in *t* - AmOH solution. With Rg_1, were obtained the 6' - O - acetyl - and 6' - O - methylmalonylderivatives 4 and 5, of which the structure has been fully determined by spectroscopic means. With Rb_1 the enzyme recognizes preferentially the terminal glucose of the disaccharide glu - O - glu moiety at C - 3OH, affording the 6" - O - acetyl - ginsenoside Rb_1 7. Its structure is suggested by NMR and MS data and substantiated by the behaviour of(20R, S) - Rg_3 8 which furnishes the 6" - O - acetylderivative 9 with the same initial reaction rate. It is expected that this enzymatic methodology will open the access to modified ginsenosides, and specifically to malonylated ginsenosides, to allow a better understanding of the structure - activity relationships for this important class of bioactive compounds.

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8. The mass spectrometer empolyed was a VG 7070EQ - HF instrument equipped with an ION TECH saddle field fast atom gun. Xenon was used to provide the high primary flux of fast atoms which was maintained at 1mA discharge current at an energy of 7KeV. Spectra were recorded at resolution about 1200, the source accelerating voltage was 6KV and scan time 500s/d. Samples were dissolved on the stainless steel target using the matrix *m* - nitrobenzylalcohol for positive spectra and diethanolamine for the negative ones. Metastable decompositions in the first FFR were recorded by B/E linked scan technique both in the spontaneous and activated(CAD) mode, with Argon as the collision gas.
9. The NMR spectra were run at 300Mz(^{1}H - and ^{13}C) on Bruker 300 EC and at 600Mz(^{1}H, homonuclear DQF - COSY, TOCSY, Heteronuclear ^{1}H - ^{13}C COSY, 2D - NOESY) on Bruker AMX - 600 instruments with approx. 0.05M solution in DMSO - d_6 at 80℃(300Mz) or 35℃(600Mz). 2D - NOESY, TOCSY and DQF - COSY(double quantum filtered) experiments were performed in phase sensitive mode, using the "noesytp", "cosydftp" and "mlevtp" pulse programs present in the instrument library, and Fourier transformed using standard procedures. TOCSY spectra were acquired using spin - lock field - strength of 10000Hz and 60 ms duration. 2D - NOESY experiments had a mixing time of 300 ms.
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