# Studies of iso-alpha-acids: analysis, purification, and stability.

Khatib, Alfi

CHAPTER 5

# Assignment of NMR Spectra of Iso-α-Acids Isolated from Isomerised Extracts of *Humulus lupulus* L. Cones

**Alfi Khatib, Erica G. Wilson, Hye Kyong Kim, Moses Supardi, Young Hae Choi, and Robert Verpoorte**

*Division of Pharmacognosy, Section of Metabolomics, Institute of Biology, Leiden University, Einsteinweg 55, PO BOX 9502, 2300 RA Leiden, The Netherlands*

**ABSTRACT**

Iso-α-acids are known to contribute to the characteristic bitter taste of beer. Six iso-α-acids were isolated from a $CO_2$ extract of the cones of *Humulus lupulus* L by centrifugal partition chromatography and β-cyclodextrin. This method overcame the low yield issue of most isolation procedures which results from the low stability of these compounds to light and oxygen. Their full identification was performed using one and two dimensional NMR spectrometry -including $^{1}$H- and $^{13}$C-NMR, $^{1}$H-$^{1}$H COSY, HMQC, and HMBC -and electrospray ionisation mass spectrometry. The results confirmed the structures of the isolated compounds as *trans*-isocohumulone, *cis*-isocohumulone, *trans*-isohumulone, *cis*-isohumulone, *trans*-isoadhumulone, and *cis*-isoadhumulone. Epimers can be well distinguished by observing the chemical shift differences of the H-5, H-1''', H-2''', and C-5 signals and the different splitting pattern of H-5 and H-2".

Submitted to *Phytochem. Anal.*, 2006

## 5.1. INTRODUCTION

Hop (*Humulus lupulus* L.) cones and their extracts are used in the beer brewing process, being largely responsible for the flavour of beer among other characteristics. The hop components that contribute to this are known generically as α-acids and consist of a mixture of three compounds: cohumulone, humulone, and adhumulone. During wort boiling they undergo isomerisation yielding iso-α-acids (IAAs) which have a very bitter taste. Their concentration in beer varies between 15 and 80 ppm (Alderweireldt *et al*., 1965; De Keukeleire *et al*., 1992; Koller, 1969; Verhagen, 1988). Each of the α-acids gives a *cis-/trans*-stereoisomeric pair of IAAs (Fig. 5.1) which have been reported to contribute to the quality of beer due to their foam lacing properties and effect on its stability (Bamforth,1985), antimicrobial activities (Sakamoto *et al*., 2001; Simpson, 1993), and final taste (Hughes *et al*., 1997; Hughes, 2000).

In view of the fact that there are actually 6 different IAAs, any further research on their contribution to the quality of beer required testing each individual IAA, therefore making it necessary to obtain the pure isomers in large amounts. Pure compounds were reported to have been prepared by photoisomerisation of humulone (Clarke and Hildebrand, 1965; Sharpe and Ormrod, 1991) and by preparative HPLC (Hughes, 1996). A mixture of all three *trans*-iso-α-acids stabilised by precipitation with dicyclohexylamine (DCHA) (Thornton *et al*., 1990; Thornton *et al*., 1993) is available commercially and is currently widely used a standard for analytical purposes. In principle this process could be used to obtain the *cis*-IAAs which do not react with DCHA, but the yield is extremely low and usually of very low purity.

Recently, we developed a simple and relatively cheap method to isolate pure α-acids and IAAs using centrifugal partitioning chromatography and β-cyclodextrin (**Chapter 4**). The identification of the compounds thus isolated had to be confirmed by spectroscopic methods.

Unfortunately there is a lack of information about NMR assignments of the IAAs. If any, this was carried out with low resolution NMR spectrometry (Borremans *et al*., 1975). There is no report available using a high resolution NMR and the absolute configuration of IAAs was determined by circular dichroism (De Keukeleire and Snatzke, 1972; De Keukeleire and Verzele, 1971).

The aim of this study therefore was to achieve the full assignment of the NMR spectra of all IAAs using high resolution NMR spectrometry.

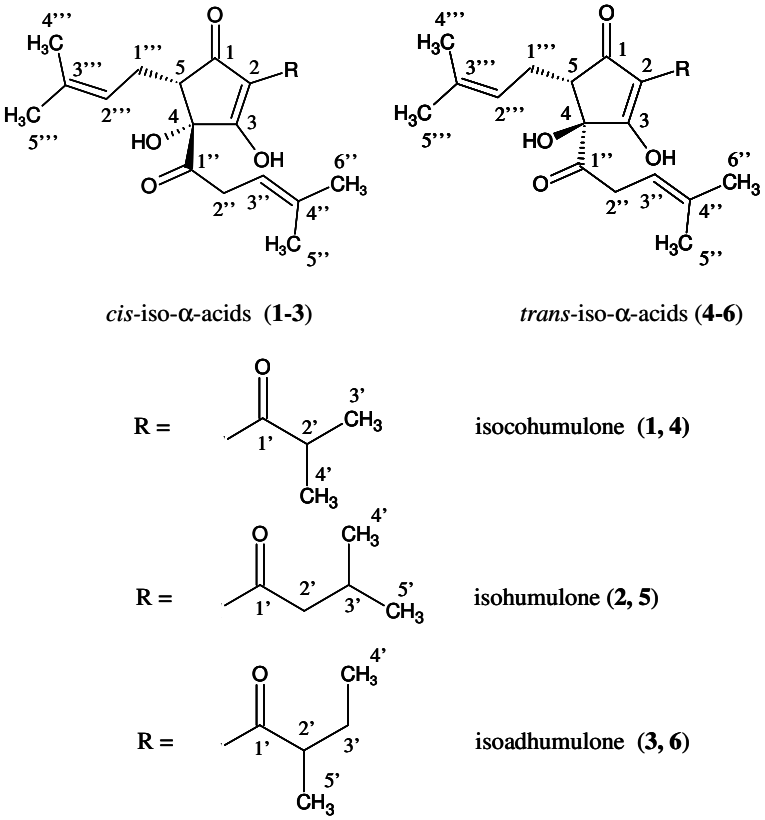


Fig. 5.1. Structures of iso-α-acids.

## 5.2. MATERIALS AND METHODS

### *5.2.1. Materials*

All organic solvents were purchased from Biosolve Co. Ltd (Valkenswaard, The Netherlands). *Ortho*-phosphoric acid 85% (w/v) was obtained from Merck (Darmstadt, Germany). β-cyclodextrin (≥ 99%) was purchased from Fluka (Steinheim, Germany). $CDCl_3$ (99.8%) was obtained from Euriso-top (Gif-Sur-Yvette, France).

A supercritical carbon dioxide hop extract was obtained from Botanix (Paddock Wood, Kent, UK).

### *5.2.2. Preparation and isolation of pure iso-α-acids*

Isolation of individual α-acids (cohumulone, humulone, and adhumulone) from a supercritical carbon dioxide hop extract was performed by centrifugal partitioning chromatography using the procedure described by Hermans-Lokkerbol and Verpoorte (1994b). The isolated α-acids were subsequently isomerized according to the method described by Koller (1969) with a small modification. It produced pairs of *trans*-/*cis*-isocohumulone, *trans*-/*cis*-iso humulone, and *trans*-/*cis*-isoadhumulone. The individual IAAs were isolated by separation of the *trans*- from its *cis*-isomers using β-cyclodextrin following the method that has been reported earlier (**Chapter 4**).

### *5.2.3. Electrospray ionisation mass spectrometry (ESI-MS)*

Spectra were recorded on a Finnigan MAT TSQ 700 triple-quadrupole mass spectrometer (Finnigan, San Jose, CA, USA) equipped with a Harvard syringe pump at a flow rate 2 μl/minute (Harvard, Kent, England), using the positive-ion modes. The applied voltage was 3000 V, and the mass scan range was 200-500 *m/z*.

*5.2.4. NMR measurements*

Forty milligrams of each pure IAA were dissolved in 1 ml $CDCl_3$. $^1H$ and $^{13}C$ NMR spectra were measured at 25 $^oC$ on a 400 MHz Bruker Avance 400 spectrometer and a 500 MHz Bruker Avance 500 spectrometer.

## 5.3. RESULTS AND DISCUSSIONS

*5.3.1. trans- and cis-Isocohumulone*

According to previous reports, IAAs have a 2-cyclopenten-1-one ring with acyl side chains at C-2 (2 methyl-1-oxopropyl), and at C-4 (4-methyl-1-oxo-3-pentenyl); an alkenyl side chain at C-5 (3-methyl-2-butenyl) (Borremans *et al*., 1975; De Keukeleire and Snatzke, 1972; De Keukeleire and Verzele, 1971).

The ESI-MS spectrum of compounds **1** and **4** isolated in this study showed *m/z* 349 $[M+H]^+$.

All the proton and carbon signals are detected well in $^1H$ and $^{13}C$ NMR spectra as shown in Table 5.1. Geminal couplings of all protons to the corresponding carbons are confirmed by HMQC spectrum. All the vicinal and geminal proton-proton couplings can be detected in the COSY spectrum as well as the long range proton-carbon couplings which are confirmed by the HMBC spectrum (Table 5.2).

$^{13}C$ NMR spectrum of compound **1** clearly showed the presence of all three ketone group at C-1 (δ 206.3), C-1' (δ 205.6), and C-1" (δ 206.9). The existence of double bonds in the alkenyl side chains is detected at C-3" (δ 114.8), C-4" (δ 136.9), C-2"' (δ 120.0), and C-3"' (δ 135.4). The up field signals are attributed to methyl carbons at C-3' (δ 18.0), C-4' (δ 18.0), C-5" (δ 18.0), C-6" (δ 25.8), C-4"' (δ 18.0), and C-5"' (δ 25.8).

The $^1H$ NMR spectrum of compound **1** shows the angular methyl signals attached to the alkenyl side chain at C-4 and C-5 at δ 1.59 (H-4"', 3H, *s*), δ 1.64 (H-5"', 3H, *s*), δ 1.57 (H-5", 3H, *s*), δ 1.72 (H-6", 3H, *s*). The singlets of these methyls are confirmed by J-resolved spectra. In the HMBC spectrum (Table 5.2), H-4"' and H-5"' are correlated to C-1"' (δ 25.7), C-2"' (δ 120.0), C-3"' (δ 135.4), and C-5 (δ 49.8). Also, H-5" and H-6" are correlated to C-2" (δ 37.0), C-3" (δ 114.8), C-4" (δ 136.9), and the

ketone carbon of C-1'' at δ 206.9. H-6'' and C-6'' resonances are more downfield than those of H-5'' and C-5'' due to the deshielding effect of the hydroxyl group of ring on C-3. A similar effect is found for H-5''' and C-5''' because they are closer to hydroxyl group of the ring at C-4 and the ketone of the C-4 side chain. The presence of hydroxyl groups is confirmed by the $^{1}$H NMR spectrum which showed broad signals at δ 4-5 and δ 9-10 belonging to hydroxyl groups at C-3 and C-4 respectively.

Other proton signals belong to angular methyls of the acyl moiety at the C-2 side chain of H-3' at δ 1.15 (3H, *d*, *J* = 6.8 Hz), and H-4' at δ 1.12 (3H, *d*, *J* = 6.8 Hz). These protons correlate to the methyne carbon (C-2' at δ 35.8) and the ketone carbon (C-1' at δ 205.6) which is confirmed by the HMBC spectrum. In the COSY spectrum, the protons of both methyls have a vicinal coupling to H-2'. The proton chemical shifts of these methyls are more up field than the ones attached to the alkenyl side chain (H-5'', H-6'', H-4''', and H-5''') because of no geminal connection to a double bond like those of the alkenyl side chain.

Other characteristic proton signals of compound **1** derive from two protons which each bind to the olefinic protons of the alkenyl side chains of H-3'' at δ 5.19 (1H, *m*) and H-2''' at δ 5.00 (1H, *m*). The correlations of H-3'' to all C-4 alkenyl side chains carbons are confirmed by HMBC. The geminal coupling of H-2''' to C-1''' and C-3''' and vicinal coupling to C-5 and the carbons of methyls of C-4''' and C-5''' at C-5 side chain are confirmed by HMBC measurement as well. The COSY spectrum confirms that H-2'''correlates to H-1'''.

This signal of a ring is detected at δ 3.20 (H-5, 1H, *t*, *J* = 5.6 Hz). The correlations of this proton to the ring ketone carbon C-1 at δ 206.3 to other ring carbons C-2 (δ 109.8), C-3 (δ 195.1), C-4 (δ 87.6), and to the alkenyl side chain carbons C-1''' (δ 25.7) and C-2'''(δ 120.0) are confirmed by the HMBC measurement. In the COSY spectrum, a vicinal coupling of H-5 is observed only to H-1'''.

Table 5.1. $^{1}H$ and $^{13}C$ chemical shift of isocohumulone stereoisomers (in $CDCl_3$, ppm).

| **Position** | ***trans*-isocohumulone** | | **Position** | ***cis*-isocohumulone** | |
|---|---|---|---|---|---|
| | **$^{1}H$ NMR** | **$^{13}C$ NMR** | | **$^{1}H$ NMR** | **$^{13}C$ NMR** |
| 1 -CO | - | 205.0 | 1 -CO | - | 206.3 |
| 2 | - | 109.2 | 2 | - | 109.5 |
| 3 -OH | - | 195.3 | 3 -OH | - | 195.1 |
| 4 -OH | - | 90.5 | 4 -OH | - | 87.6 |
| 5 | 3.06 (1H, *dd* , *J* = 9.8 Hz, *J* = 6.0 Hz) | 54.8 | 5 | 3.20 (1H, *t*, *J* = 5.6 Hz) | 49.8 |
| 1'-CO | - | 203.4 | 1'-CO | - | 205.6 |
| 2' | 3.45 (1H, *m*) | 34.6 | 2' | 3.50 (1H, *m*) | 35.8 |
| 3' -Me | 1.17 (3H, *d, J* = 6.9 Hz) | 18.0 | 3' -Me | 1.15 (3H, *d, J* = 6.8 Hz) | 18.0 |
| 4' -Me | 1.12 (3H, *d, J* = 6.9 Hz) | 18.0 | 4' -Me | 1.12 (3H, *d, J* = 6.8 Hz) | 18.0 |
| 1" -CO | - | 206.9 | 1" -CO | - | 206.9 |
| 2" | 3.31 (2H, *m*) | 38.8 | 2" | 3.30 (2H, *d*, *J* = 6.9 Hz) | 37.0 |
| 3" | 5.20(1H, *m*) | 114.5 | 3" | 5.19 (1H, *m*) | 114.8 |
| 4" | - | 136.2 | 4" | - | 136.9 |
| 5"-Me | 1.56 (3H, *s*) | 18.0 | 5"-Me | 1.56 (3H, *s*) | 18.0 |
| 6"-Me | 1.73 (3H, *s*) | 25.7 | 6"-Me | 1.70 (3H, *s*) | 25.8 |
| 1'''a | 2.57 (1H, *m*) | 23.4 | 1''' | 2.43 (2H, *m*) | 25.7 |
| b | 2.32 (1H, *m*) | | | | |
| 2''' | 5.12 (1H, *m*) | 120.1 | 2''' | 5.00 (1H, *m*) | 120.0 |
| 3''' | - | 134.8 | 3''' | - | 135.4 |
| 4''' -Me | 1.53 (3H, *s*) | 18.0 | 4''' -Me | 1.58 (3H, *s*) | 18.0 |
| 5''' -Me | 1.68 (3H, *s*) | 25.7 | 5''' -Me | 1.62(3H, *s*) | 25.8 |

Table 5.2. HMBC correlation in *cis*-isocohumulone.

| **Proton and chemical shift (ppm)** | **Correlation to carbon and chemical shift (ppm)** |
|---|---|
| H-5(3.20) | C-1(206.3), C-2(109.8), C-3(195.1), C-4(87.6), C-1’’’(25.7), C-2’’’(120.0) |
| H-2’(3.50) | C-1’(205.6), C-3’(26.3), C-4’(11.4) |
| H-3’(1.15) | C-1’(205.6), C-2’(35.8), C-4’(18.0) |
| H-4’(1.12) | C-1’(205.6), C-2’(35.8), C-3’(18.0) |
| H-2’’(3.30) | C-1’’(206.9), C-3’’(114.8), C-4’’(136.9) |
| H-3’’(5.19) | C-1’’(206.9), C-2’’(37.0), C-4’’(136.9), C-5’’(18.0), C-6’’(25.8) |
| H-5’’(1.56) | C-1’’(206.9), C-2’’(37.0), C-3’’(114.8), C-4’’(136.9), C-6’’(25.8) |
| H-6’’(1.70) | C-1’’(206.9), C-2’’(37.0), C-3’’(114.8), C-4’’(136.9), C-5’’(18.0) |
| H-1’’’(2.43) | C-1(206.3), C-4(87.6), C-5 (49.8), C-2’’’(120.0), C-3’’’(135.4) |
| H-2’’’(5.00) | C-5 (49.8), C-1’’’(25.7), C-3’’’(135.4), C-4’’’(18.0), C-5’’’(25.8) |
| H-4’’’(1.58) | C-5 (49.8), C-1’’’(25.7), C-2’’’(120.0), C-3’’’(135.4), C-5’’’(25.8) |
| H-5’’’(1.62) | C-5 (49.8), C-1’’’(25.7), C-2’’’(120.0), C-3’’’(135.4), C-4’’’(18.0) |

Compounds **1** and **4** can be distinguished by the chemical shift of the H-5 and C-5 signals. The proton signal of compound **1** at δ 3.20 (H-5, 1H, *t*, *J* = 5.6 Hz) is more downfield than δ 3.06 of compound **4** (H-5, 1H, *dd*, *J* = 9.8 Hz, *J* = 6.0 Hz) because it is closer to the ketone of C-1". However, the carbon signal of compound **1** at this position (δ 49.8) is more up field than that of compound **4** (δ 54.8) due to a further distance to the ketone of C-1".

Another difference of the epimers is the difference of the proton chemical shift of methylene (H-1'") at the C-5 side chain. The methylene protons of the *trans*-isomer (H-1'"a and H-1'"b) are not equivalent due to a different distance to the ketone at C-1", which is not the case in the *cis*-isomer. It affects the splitting pattern of the H-5 signal which is a double doublet for that of the *trans*-isomer and triplet for that of the *cis*-isomer. In the HMBC spectrum, the protons of this methylene had a geminal coupling to C-5 and C-2'", and a vicinal coupling to C-3'" and the carbon of the ring ketone (C-1). The vicinal coupling of these methylene proton to H-5 and H-2'" is confirmed by COSY measurements.

A difference for the epimers is also noticed in the H-2'" chemical shift, which is more downfield for the *trans*-isomer than for the *cis*-isomer because the H-2'" of the *trans*-isomer is closer to the ketone at C-1".

The last difference between the epimers is the splitting pattern of the H-2" signal. The H-2" signal of the *trans*-isomer is a multiplet. But this is not the case for those of the *cis*-isomer due to the free rotation of the H-2".

### *5.3.2. trans- and cis-Isohumulone*

The difference between the structure of compounds **2** and **5** and that of compounds **1** and **4** is the moiety at C-2 side chain (3-methyl-1-oxobutyl). ESI-MS spectra of the epimers exhibited 363 *m/z* as $[M+H]^+$ peak indicating the additional 14 *m/z* due to the methylene group at the C-2 side chain when compared to that of compounds **1** and **4**.

$^{1}H$ and $^{13}C$ NMR chemical shifts of compounds **2** and **5** are shown in Table 5.3. Most of the proton and carbon chemical shifts of these compounds are similar to those of compounds **1** and **4**, except those at the C-2 side chain.

The proton chemical shifts of the angular methyls (H-4' and H-5') at the C-2 side chain of these compounds are more up field than those of compounds **1** and **4** due to a longer distance to the ketone at C-1'. In HMBC, the protons of these methyls are

coupled to C-2' and C-3' as shown in Table 5.4. Vicinal coupling to H-3' is confirmed by the COSY spectrum.

In the $^{1}$H NMR spectra, H-2' appeared as a doublet due to a vicinal coupling to only one proton (H-3'). This coupling is confirmed by COSY measurement. In the HMBC spectrum, the connection of these protons to C-3', the ketone carbon (C-1'), and the carbons of angular methyls (C-4' and C-5') are confirmed as well.

The differences in the NMR spectrum of the epimers are similar to those of compounds **1** and **4** as mentioned above. No differences for the C-2 side chain are observed between the NMR spectra of the epimers.

### *5.3.3. trans- and cis-Isoadhumulone*

ESI-MS spectrum of compounds **3** and **6** showed $[M+H]^+$ peak at *m/z* 363 which is similar to those of compounds **2** and **5**.

These compounds differ from compounds **2** and **5** in a 2-methyl-1-oxobutyl moiety at C-2. The proton signals from two methyls of this moiety are not overlapping as those in other IAAs (Table 5.5). H-5' signal chemical shift is more downfield when compared to that of H-4' because H-5' is closer to the ketone of C-1'. The proton signal splitting pattern of both methyls is in accordance with the chemical structure. Following the first-order splitting pattern, the H-5' signal appears as a doublet due to one vicinal proton (H-2'), whereas H-4' signal is a triplet because it has two vicinal protons (H-3'). In the HMBC spectrum (Table 5.6), H-4' connects to C-3' and C-2' but not to the ketone carbon (C-1'). A connection to the ketone carbon is detected for the closer methyl proton (H-5').

Table 5.3. $^{1}H$ and $^{13}C$ chemical shift of isohumulone stereoisomers (in $CDCl_3$, ppm).

| Position | *trans*-isohumulone | | Position | *cis*-isohumulone | |
|---|---|---|---|---|---|
| | $^{1}H$ NMR | $^{13}C$ NMR | | $^{1}H$ NMR | $^{13}C$ NMR |
| 1 -CO | - | 204.9 | 1 -CO | - | 206.3 |
| 2 | - | 110.3 | 2 | - | 110.7 |
| 3 -OH | - | 195.6 | 3 -OH | - | 195.6 |
| 4 -OH | - | 90.7 | 4 -OH | - | 87.7 |
| 5 | 3.02 (1H, *dd* , *J* = 9.7 Hz, *J* = 6.0 Hz) | 55.3 | 5 | 3.19 (1H, *t*, *J* = 5.6 Hz) | 50.2 |
| 1'-CO | - | 197.8 | 1'-CO | - | 200.6 |
| 2' | 2.70 (2H, *d*, *J* = 6.7 Hz) | 44.4 | 2' | 2.72 (2H, *d*, *J* = 6.9 Hz) | 46.0 |
| 3' | 2.14 (1H, *m*) | 26.5 | 3' | 2.13 (1H, *m*) | 26.0 |
| 4' -Me | 0.97 (3H, *d*, *J* = 6.6 Hz) | 22.5 | 4' -Me | 0.96 (3H, *d*, *J* = 6.5 Hz) | 22.7 |
| 5' -Me | 0.95 (3H, *d*, *J* =7.4 Hz) | 22.5 | 5' -Me | 0.95 (3H, *d*, *J* = 6.0 Hz) | 22.7 |
| 1" -CO | - | 206.9 | 1" -CO | - | 207.0 |
| 2" | 3.30 (2H, *m* ) | 38.7 | 2" | 3.29 (2H, *d* , *J* = 5.9 Hz) | 37.1 |
| 3" | 5.17 (1H, *m*) | 114.4 | 3" | 5.19 (1H, *m*) | 114.8 |
| 4" | - | 136.2 | 4" | - | 136.8 |
| 5"-Me | 1.55 (3H, *s*) | 18.0 | 5"-Me | 1.56 (3H, *s*) | 18.4 |
| 6"-Me | 1.72 (3H, *s*) | 25.6 | 6"-Me | 1.72 (3H, *s*) | 26.0 |
| 1'''a | 2.56 (1H, *m*) | 23.3 | 1''' | 2.42(2H, *m*) | 25.6 |
| b | 2.31 (1H, *m*) | | | | |
| 2''' | 5.12 (1H, *m*) | 120.1 | 2''' | 4.99 (1H, *m*) | 119.9 |
| 3''' | - | 134.6 | 3''' | - | 135.3 |
| 4''' -Me | 1.53 (3H, *s*) | 18.0 | 4''' -Me | 1.58 (3H, *s*) | 18.0 |
| 5''' -Me | 1.68 (3H, *s*) | 25.6 | 5''' -Me | 1.63 (3H, *s*) | 26.0 |

Table 5.4**.** HMBC correlation in carbonyl moiety at C-2 side chain (3-methyl-1-oxobutyl) of *cis*-isohumulone.

| Proton and chemical shift (ppm) | Correlation to carbon and chemical shift (ppm) |
|---|---|
| H-2’ (2.72) | C-1’(200.6), C-3’(26.0), C-4’(22.7), C-5’(22.7) |
| H-3’ (2.13) | C-1’(200.6), C-2’(46.0), C-4’(22.7), C-5’(22.7) |
| H-4’ (0.96) | C-2’(46.0), C-3’(26.0), C-5’(22.7) |
| H-5’ (0.95) | C-2’(46.0), C-3’(26.0), C-4’(22.7) |

The methylene protons (H-3'a and b) of this moiety have non equivalent chemical shifts due to a non symmetrical structure. One of the proton signal is overlapped with the H-6” methyl signal and the other signal is in the less crowded up field area. In the HMBC spectrum, the connection of H-3'a and H-3'b to the ketone carbon of C-1', C-2' and methyl carbons of C-4' and C-4' are confirmed (Table 5.6). The vicinal coupling of both protons to H-4' and H-2' are also detected by the COSY measurement.

The other NMR signals of compounds **3** and **6** are similar to those of other IAAs. Thus the difference between epimers can be noticed in the same way as mentioned above.

Hughes (2006) reported the presence of different tautomers of iso-α-acids. Three of the 19 tautomers were calculated the most stable tautomers. Considering their structures and the NMR spectra in chloroform in our investigation, it seems that the keto-keto-enol (KKE) is the most abundant with a clearly visible H-5 and no singlet for H-2. However, several minor signals may represent some of the proposed tautomers.

Table 5.5. $^{1}H$ and $^{13}C$ chemical shift of isoadhumulone stereoisomers (in $CDCl_3$, ppm).

| Position | *trans*-isoadhumulone | | Position | *cis* -isoadhumulone | |
|---|---|---|---|---|---|
| | $^{1}H$ NMR | $^{13}C$ NMR | | $^{1}H$ NMR | $^{13}C$ NMR |
| 1 -CO | - | 205.4 | 1 -CO | - | 206.3 |
| 2 | - | 109.8 | 2 | - | 109.8 |
| 3 -OH | - | 195.6 | 3 -OH | - | 195.4 |
| 4 -OH | - | 90.8 | 4 -OH | - | 87.5 |
| 5 | 3.02 (1H, *dd, J* = 9.8 Hz, *J* = 6.0 Hz) | 55.2 | 5 | 3.21 (1H, *dd* , *J* = 7.3 Hz, *J* = 5.8 Hz) | 49.9 |
| 1'-CO | - | 203.0 | 1'-CO | - | 204.9 |
| 2', 2'' | 3.33 (3H, *m*) | 41.0, 38.9 | 2', 2'' | 3.34 (3H, *m*) | 41.7, 36.8 |
| 3' a | 1.67 (1H, *m*) | 23.6 | 3' a | 1.72 (1H, *m*) | 26.3 |
| b | 1.48 (1H, *m*) | | b | 1.43 (1H, *m*) | |
| 4' -Me | 0.92 (3H, *t, J* = 7.5 Hz) | 11.7 | 4' -Me | 0.92 (3H, *t, J* = 7.4 Hz) | 11.4 |
| 5' -Me | 1.12 (3H, *d, J* = 6.8 Hz) | 15.7 | 5' -Me | 1.12 (3H, *d, J* = 6.8 Hz) | 15.1 |
| 1'' -CO | - | 205.4 | 1'' -CO | - | 206.3 |
| 3'' | 5.19 (1H, *m*) | 114.6 | 3'' | 5.22 (1H, *m*) | 114.6 |
| 4'' | - | 136.4 | 4'' | - | 136.7 |
| 5''-Me | 1.57 (3H, *s*) | 18.3 | 5''-Me | 1.57 (3H, *s*) | 17.9 |
| 6''-Me | 1.73 (3H, *s*) | 26.0 | 6''-Me | 1.72 (3H, *s*) | 25.7 |
| 1'''a | 2.58 (1H, *m*) | 26.6 | 1'''a | 2.48 (1H, *m*) | 25.6 |
| b | 2.32 (1H, *m*) | | b | 2.42 (1H, *m*) | |
| 2''' | 5.13 (1H, *m*) | 120.4 | 2''' | 5.01 (1H, *m*) | 119.6 |
| 3''' | - | 134.9 | 3''' | - | 135.2 |
| 4''' -Me | 1.53(3H, *s*) | 18.3 | 4''' -Me | 1.59 (3H, *s*) | 17.9 |
| 5''' -Me | 1.69 (3H, *s*) | 26.0 | 5''' -Me | 1.64 (3H, *s*) | 25.7 |

Table 5.6. HMBC correlation in carbonyl moiety at C-2 side chain (2-methyl-1-oxobutyl) of *cis*-isoadhumulone.

| Proton and chemical shift (ppm) | Correlation to carbon and chemical shift (ppm) |
|---|---|
| H-2' (3.34) | C-1'(204.9), C-3'(26.3), C-4'(11.4), C-5'(15.1) |
| H-3'a (1.72) | C-1'(204.9), C-2'(41.7), C-4'(11.4), C-5'(15.1) |
| H-3'b (1.43) | C-1'(204.9), C-2'(41.7), C-4'(11.4), C-5'(15.1) |
| H-4' (0.92) | C-2'(41.7), C-3'(26.3) |
| H-5' (1.12) | C-1'(204.9), C-2'(41.7), C-3'(26.3) |

It can be concluded that the assignment of IAAs in this study is in the agreement with the structure of IAAs which have been previously reported (Borremans *et al*., 1975; De Keukeleire and Snatzke, 1972; De Keukeleire and Verzele, 1971) although the ketone carbons at C-4 side chain (C-1") and at ring (C-1), could not be detected by $^{13}C$ NMR in those report, except for the one in C-2 side chain. Therefore, the existence of these ketone carbons are for the first time confirmed by our work.