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THE ABSOLUTE CONFIGURATION OF THE ISOHUMULONES AND THE HUMULINIC ACIDS

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Abstract—The absolute configurations for the *trans* and *cis* diastereoisometric isohumulones (II) and humulinic acids are determined (III). This is achieved mainly by reductive transformation of III into the dihydrodeoxohumulinic acids (IV), and application of the Horeau method. *Trans* isohumulone is the S(C-4) S(C-5) form and *cis* isohumulone the R(C-4) S(C-5) form.

TRANS AND *cis* isohumulones (II) are formed during isomerization of (−)-humulone (I) in the brewing process; they give beer the bitter taste. On alkaline treatment, they are degraded to the humulinic acids (III) (Fig. 1). *Trans* refers to the trans position of the OH function at C_4 and the alkenyl chain at C_5, the same applies to *cis*. The relative configuration of these compounds is indeed well known.[1] While the absolute configuration of (−)-humulone was determined by us before,[2] that of the diastereoisomeric isohumulones and humulinic acids was still unknown. The two chiral centra are located in the five-membered ring at C_4 and C_5.

The key product: 2,5-*di*-(3′-*methyl*)*butyl*-4-*oxycyclopentane*-1,3-*dione* [*dihydro-deoxo-humulinic acid* (IV)].

The absolute configuration of (+)-*cis*-isohumulone and (+)-*cis*-humulinic acid is the same, and such is the case for the corresponding *trans*-compounds. So, we can use the humulinic acids as they are the easiest obtainable compounds[3] in this series. However one of the difficulties is that the enolization pattern of the β-tricarbonylsystem is not fixed in these substances. This was overcome by modifing the humulinic acid structure to a dicarbonyl system by hydrogenolysis, fixing the two possible enolic forms as methyl enol ethers and separating. The known hydrogenolysis reaction,[4] was described with optically inactive humulinic acid and produced very low yields (10–15%). Optically active humulinic acids with which we intended to do the reaction have only been known for a short time.[5] The question was however if the reaction sequence of Fig. 1 would be possible while maintaining optical activity, as the hydrogenolysis is carried out in strong acid medium and this could cause racemization. The reaction was therefore studied in detail.[6] The yield of hydrogenolysis products could be increased to 80–85% while maintaining at least partial optical activity. By carring out the reaction on 20% weight concentration of humulinic acid in AcOH with 5% Adams' catalyst and with electrolytic hydrogen at 55° for 6–8 hr.

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The dihydrodeoxohumulinic acids were separated by counter-current distribution (CCD) and treated with a slight excess of CH_2N_2. The resulting methyl enol ethers can be separated by GC and also by CCD. An appropriate phase system, is isooctane (upper phase) and a mixture of 60 volume % EtOH and 40 volume % water (lower phase). The distributions were carried out with the recyclization procedure and were repeatedly stopped to check the degree of separation. About 2500 transfers were necessary for full separation. The products with the lower K-value prevail in each case for ±85%.

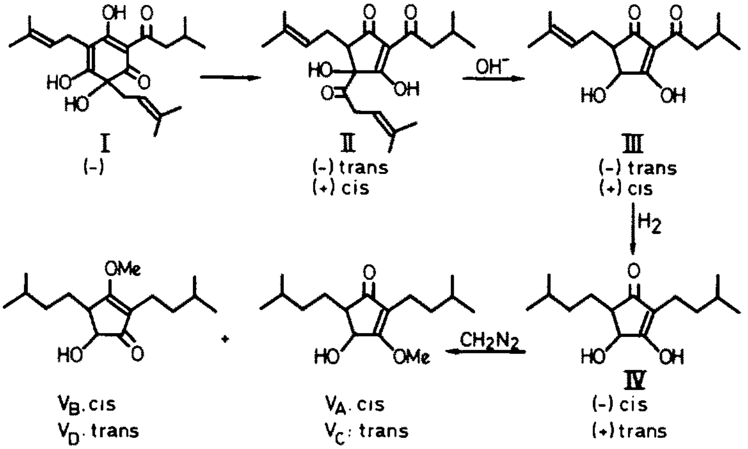

FIG. 1. Chemical transformations of humulone (I) as discussed in this paper

Individual distinction between the isomers is made with the “enone rule”.[7] This rule states that a hydrogen atom next to the carbonyl function of an enolized β-di carbonyl system is found at higher field in the NMR spectrum that a hydrogen atom next the double bond of the enolic function. The respective δ values for the hydrogen atom at the chiral centre carrying the alcoholic function are 4·81 and 4·12 in the *cis*-series, 4·38 and 3·69 in the *trans*-series. The major products are recognized as 2,5-di-(3′-methyl)butyl-3-methoxy-4-hydroxy-2-cyclopentenones, the minor ones being 2,4-di-(3′-methyl)butyl-3-methoxy-5-hydroxy-2-cyclopentenones. All the substances are still optically active.

The relative configuration of the dihydrodeoxohumulinic acids

The *cis* and *trans* enol ethers are diastereoisomers and one of the two chiral centres must thus be the same. In principle we can find out which by removing one chiral centre and by checking if the resulting products have similar or mirror ORD curve. This can be achieved in two ways. Either by removal of the OH group by prolonged hydrogenolysis or by oxidation of the same OH to a carbonyl function. In each case the substances derived from *trans* or *cis* dihydro deoxohumulinic acid were identical and showed optical activity with the same ORD curve. In the hydrogenolysis, yields up to 30% of 2,5-di(3′-methyl)butyl-cyclopentane-1,3-dione were obtained and the optical activity $[\alpha_D]$ was as high as $-10°$. It is unexpected that this substance is not racemized completely in the strong acid medium of the hydrogenolysis reaction but this fact was checked repeatedly. Frankly unbelievable at first was that the dialkyl triones obtained by bismuth oxide oxidation of IV still had some, although weak, optical activity. This was however also confirmed. The explanation that can be advanced is that enolization is complete in this substance and that racemization involves either a second double bond or a second negative charge in the five-membered ring, both very unfavourable propositions. The conclusion of all this is that the chiral centre bearing the OH group must thus necessarily be enantiomeric in the *cis* and *trans* series under discussion. This is also proved directly from the results with Horeau's method (see below).

The absolute configuration of dihydrodeoxohumulinic acids

The Horeau method of partial decoupling[8] was applied to the four enol ethers with and without hydrolysis. Fig 2 shows the ORD curves of the isolated 2-phenylbutanoic acids,

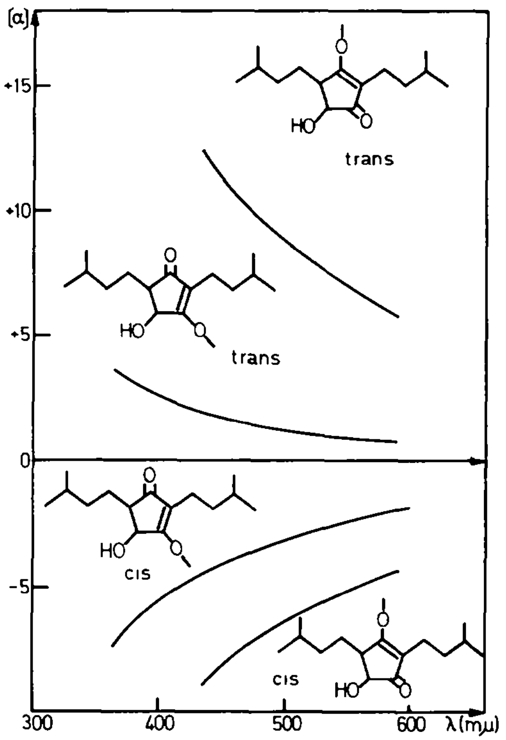

FIG. 2. Rotation of the phenylbutanoic acid as isolated in the Horeau procedure

resulting from the procedure with hydrolysis. The roatation at the sodium-D line of the reaction mixture in the method without hydrolysis gives the same indications. The yields and optical yields of the four enol ethers VA to VD are summarized in the following table.

The yields of the esterification are lower and the optical yields higher in the *cis*-series, as could be expected for these sterically more hindered substances.

In VA and VC the steric hindrance competition is between an alkyl and a OMe function, while it is between alkyl and carbonyl in VB and VD. The difference in steric hindrance is more marked in the latter and the optical yields are therefore higher. It is

Compound	Ester yield %	Optical yield %	Rotation sign
cis-VA	68	5·1	—
cis-VB	64	11·7	—
trans-VC	100	1·1	+
trans-VD	100	9·9	+

Yield of esterification with racemic 2-phenylbutanoic acids, optical yield of optically active 2-phenylbutanoic acid (method of Horeau).

lowest in VC as would be expected. These facts are in agreement with the structural assignments and also lead to the conclusion that the alkyl group is the large substituent (L) and the other side the medium (M) one. The *cis*-derivatives yield R(−)-2-phenylbutanoic acid, while the *trans*-compounds produce S(+)-2-phenylbutanoic acid. This is direct proof that the chiral centre carrying the hydroxylic function is of opposite absolute configuration. To apply Horeau's rule, one must draw the Fisher projections around the chiral centre with the OH function on the left and the hydrogen atom on the right (Fig 3). In the case where R(−)-2-phenylbutanoic acid was formed, the large group (L) must be upwards, leading to the correct absolute structures for the *cis* compounds.

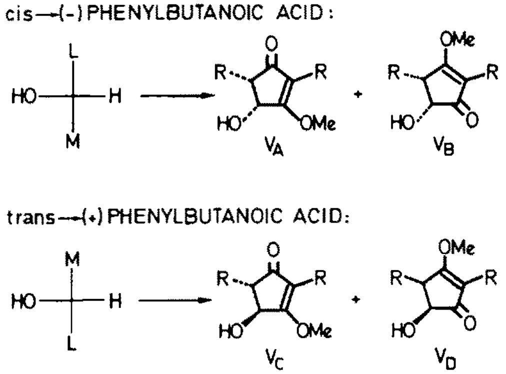

FIG. 3. Correlation of Fisher projection according to Horeau and the absolute configuration of the methyl ethers of dihydrodeoxohumulinic acids

Fig 3 also shows the parallel treatment for the *trans*-series. Consequently, the 3-methylbutyl side chain takes the S-configuration in each case, the secondary alcohol chiral centre is the R form in the *cis*- and the S form in the *trans*-series.

The absolute configuration of the isohumulones and the humulinic acids

The absolute representation of the methyl enol ethers can directly be transposed to the humulinic acids and the isohumulones. As published elsewhere,[9] the CD spectra of all the substances mentioned in this paper, are in full agreement with the established absolute configurations. Since we now know the absolute configuration of the isohumulones and also that of (−)-humulone, it is possible to deduce the stereochemical course of the isomerization mechanism of (−)-humulone. This is shown in Fig 4. The proton attack is such that in the intermediate ketonized anion the two alkenyl side chains take the *trans*-position.

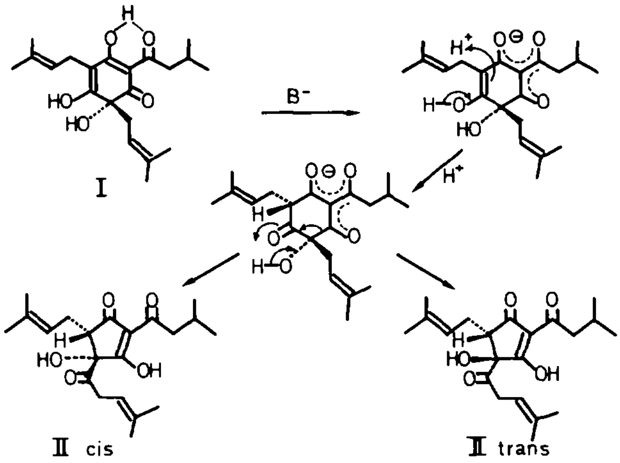

FIG. 4. Isomerization mechanism of humulone

EXPERIMENTAL

All CCD separations were carried out with a 400 cell CCD apparatus, built in the laboratory work-shop. The NMR spectra are recorded with a Varian HA 60D spectrometer in 10% CCl_4 solutions with TMS as internal reference. The ORD spectra taken in MeOH (only at 5 different wave lengths: 589, 578, 546, 436 and 365 nm) were obtained with a Perkin-Elmer 141 polarimeter. The thin-layer chromatograms are analysed with a chromatogram-spectral photometer Zeiss PMQ II.

Preparation of dihydrodeoxohumulinic acids. 2 g Humulinic acid was dissolved in 10 g AcOH and 5% Adams' catalyst added. The hydrogenation vessel was modified for reaction at a constant temp. (55°). The reaction was followed by TLC on silicagel coated plates with ether/C_6H_6 3/1. After 6 to 8 hr the catalyst was filtered off and the solvent evaporated. The *trans* and *cis* dihydrodeoxohumulinic acids mixtures were separated by CCD in the two phase system: ether/0·25 M phosphate buffer pH = 7·65, over 100 transfers; the *cis* compound (m.p. 190°) has a K-value of 1·36, the *trans* isomer 1·23 (m.p. 179°). For spectroscopic properties refer to another paper in this series.[6]

The methyl enol ethers of the dihydrodeoxohumulinic acids. Cis- and *trans*-Dihydrodeoxohumulinic acids were separately treated with a slight excess of CH_2N_2; after.1 hr the solvent was evaporated and the residue separated in the two isomeric enol ethers by GC or by CCD. The preparative CCD isolation was carried out in the two phase system iso-octane as upper phase and a mixture of Et_2O/H_2O 3/2 as lower layer. In this case the mean K value was around 0·6 and the β-value ± 1·2. All four enol ethers are light yellow oils with the UV absorption maxima situated around 245 nm.

(+) *cis-2,4-Di(3'-methyl)butyl-3-methoxy-5-hydroxy-2-cyclopentenone* (VB). Mass spectrum: m+ = 268; the main peak at *m/e* 43; other important peaks at *m/e* 253, 198, 142, 121, 119, 117, 58 and 41.

ORD spectrum: 589 nm: + 29·5°; 578 nm: + 31·6°; 46 nm: + 37·5°; 436 nm: + 75·6°; 365 nm: + 120°.

NMR: two triplets around $\delta = 0{\cdot}85$ and $\delta = 0{\cdot}87$ ($J = 6$ cps) owing to different isopropyl groups; a multiplet between δ = 1 to 2 (relative area: 9 H) for the methylene and methine protons of the alkyl chains; $\delta = 2{\cdot}11$: triplet for the methylene function next to the double bond; $\delta = 3{\cdot}0$: —OH function; $\delta = 3{\cdot}98$: OMe; δ = 4·12: doublet for the proton at the secondary alcohol functions (J = 7 cps: *cis*-coupling).

(−) *cis-2,5-Di-(3'-methyl)butyl-3-methoxy-4-hydroxy-2-cyclopentenone* (VA). Mass spectrum: $m^+ = 268$; the most important peak at *m/e* 41; other peaks at *m/e* 253, 212, 198, 195, 155, 124, 119, 117, 95, 69, 57, 55 and 43.

ORD spectrum: 589 nm: −26·3°; 578 nm: −27·2°; 546 nm: −29·4°; 436 nm: −26·5°; 365 nm: + 137·9°.

NMR: δ=0·79 and δ=0·80: two triplets for two discernible isopropyl groups; between δ=1 and 2: multiplet (relative area: 9 H) caused by the methylene and methine protons in the side chains; δ=2·01: triplet for the methylene function next to the double bond; δ=3·18: —OH grouping; δ=4·08: OMe; δ=4·81: doublet (J=7 cps: *cis*-coupling) for the proton at C_4.

(+) *trans*-2,4-*Di*-(3′-*methyl*)*butyl*-3-*methoxy*-5-*hydroxy*-2-*cyclopentenone* (VD). Mass spectrum: m^+=268; the main peak is *m/e* 212 and other peaks are found at *m/e* 253, 197, 179, 169, 156, 155, 142, 141, 138, 124, 123. III, 109, 97, 95, 83, 81, 79, 77, 69, 67, 57, 55, 43 and 41.

ORD spectrum: 589 nm: + 22·79°; 578 nm: + 24·25°; 546 nm: + 28·85°; 436 nm: + 60·73°; 365 nm: + 142·16°.

NMR: δ=0·88: doublet integrating for two isopropyl groups; δ=1 to 2: multiplet with a relative area of 9 H for the protons of the side chains; δ=2·2: triplet of the methylene function next to the double bond; δ=2·6: —OH function; δ=3·69: doublet for the next to OH proton (J=2·5 cps: *trans*-coupling) δ=3·98: OMe.

(+)-*trans*-2,5-*Di*-(3′-*methyl*)*butyl*-3-*methoxy*-4-*hydroxy*-2-*cyclopentenone* [*VC*]. Mass spectrum: m^+=268; the main peak at *m/e* 198 and important fragments at *m/e* 253, 212, 211, 199, 142, 141, 123, 111, 110, 109, 95, 81, 69, 67, 37, 55, 43, and 41.

ORD spectrum: 589 nm: + 11·53°; 578 nm: + 12·27°; 546 nm: + 13·62°; 430 nm: + 18·04°; 365 nm: −20·98°.

NMR: δ=0·87: two isopropyl moieties; δ=1 to 2: multiplet of the methylene and methine protons of the alkyl chains; δ=2·02: triplet for the methylene function next to the double bond; δ=2·9: —OH function; δ=4·L: OMe; δ=4·38: doublet for the hydrogen atom at the secndary hydroxyl function (J=2·5 cps: *trans*-coupling).

Bismuth oxide oxidation of the dihydrodeoxohumulinic acids. 300 mg Optically active *cis*- or *trans*-dihydrodeoxohumulinic acid was heated in 25 ml AcOH for 5 hr. The solution was poured out into ice-chilled HCl 2N. and subsequently extracted with ether. The ether fractions were washed with 10 ml H_2O and dried (Na_2SO_4). After evaporating the solvent, the residue was separated by CCD in the two phase system: ether/0·25 m phosphate buffer pH=8·6 over 100 transfers with a K value around 0·9.

The method of partial decoupling (*method of Horeau*) *with hydrolysis. General procedure.* ± 10^{-4} mol Enol ether (25–30 mg) and 0·5 ml pyridine solution containing ± 2·10^{-4} mol (60–70 mg) 2-phenyl-butanoic acid anhydride were mixed. After standing for 15 to 20 hr at room temp., a few drops of H_2O were added, followed by heating on a steam bath for 30 min. The solution was added to 2 ml H_2O and 3 ml C_6H_6 and titrated with 0·1 N NaOH on phenolphthalein. After adding 10 ml C_6H_6, the water layer was extracted twice more, this layer was acidified with 2 ml HCl. Finally the optical rotation of the formed 2-phenylbutanoic acid was measured.

(−)-*cis*-2,5-*Di*-(3′-*methyl*)*butyl*-3-*methoxy*-4-*hydroxy*-2-*cyclopentenone* (VA). Following α values were measured: 589 nm: −2·03°; 578 nm: −2·15°; 546 nm: −2·41°; 436 nm: −4·5°; 365 nm: −7·39°.

(+)-*cis*-2,4-*Di*-(3′-*methyl*)*butyl*-3-*methoxy*-5-*hydroxy*-2-*cyclopentenone* (VB). α Values of the isolated acid were: 589 nm: −4·4°; 578 nm: −47°; 546 nm: −5·35°; 436 nm: −9°.

(+)-*trans*-2,5-*Di*-(3′-*methyl*)*butyl*-3-*methoxy*-4-*hydroxy*-2-*cyclopentenone* (VC). The measured α values were: 589 nm: +0·62°; 578 nm: +0·77°; 546 nm: +0·93°, 436 nm: + 1·95°; 365 nm: + 3·59°.

(+)-*trans*-2,4-*Di*-(3′-*methyl*)*butyl*-3-*methoxy*-5-*hydroxy*-2-*cyclopentenone* (VD). The measured α values were: 589 nm: + 5·86°; 578 nm: + 6·11°; 546 nm: + 7·02; 436 nm: + 12·4°.

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