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THE STRUCTURE AND THE ABSOLUTE CONFIGURATION OF (−)HUMULONE

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Abstract When (−)humulone is forced to absorb seven moles of hydrogen (catalytic hydrogenation), the principal product (40%) is 2,4-di-(3-methyl)butyl-6-(3-methyl)-butanoyl-resorcinol. This fact and other evidence indicates that the correct enol form of (−)humulone is II in Chart I. Interpretation of the ORD curve of (−)humulone in accordance with this enol structure, establishes that this natural substance has the *R*-configuration and belongs to the *L*-series. Ozonization experiments on (−)tetrahydrohumulone confirm this result.

Humulone (I), the main bitter acid of hops, occurs in nature in optically active form. It has one chiral centre of unknown absolute configuration and shows leavorotation in the acid form.

Howard[1] tentatively attributed the D-configuration to (−)humulone by analogy with the D-2-hydroxycarboxylic acids. In fact the salts of these acids, like the salts of (−)humulone,[2] turn the plane of plain polarized light more to the right than do the free acids. As this was only a suggestion by Howard, the absolute configuration of (−)humulone is the subject of this paper.

ORD theory and the octant rule[3] can only be applied to (−)humulone if the position of the carbonyl chromophore in the humulone ring and the enolization situation of the β-tricarbonylchromophore is known. Usually humulone is represented by structure I in chart I. There is, however, no reason to believe that this is the correct enolization form, and indeed we have proved that the correct enol form is structure II in chart I. This was deduced from perhydrogenation studies of (−)tetrahydrohumulone. Knowing the exact formulation of humulone, the ORD curve could be interpreted and this led to the conclusion that humulone belongs to the L series and has the R configuration. This configuration was confirmed by the isolation of a substance with known absolute configuration from the ozonolysis of (−)tetrahydrohumulone.

A. *Perhydrogenation of (−)tetrahydrohumulone*

(−)Tetrahydrohumulone was prepared as previously described.[4,5] After purifying the compound by adsorption chromatography on silicagel with benzene as eluent, it solidified in the freezer (m.p. 58·5–59·5°). Although (−)tetrahydrohumulone is described as an oil, racemic tetrahydrohumulone, prepared by hydrogenolysis of lupulone, melts at 84°.[6] The double bonds in the 3-methyl-2-butenyl side chains of humulone first have to be saturated to prevent hydrogenolysis during the perhydrogenation in acid medium with activated Adam's catalyst. Perhydrogenation of tetra-

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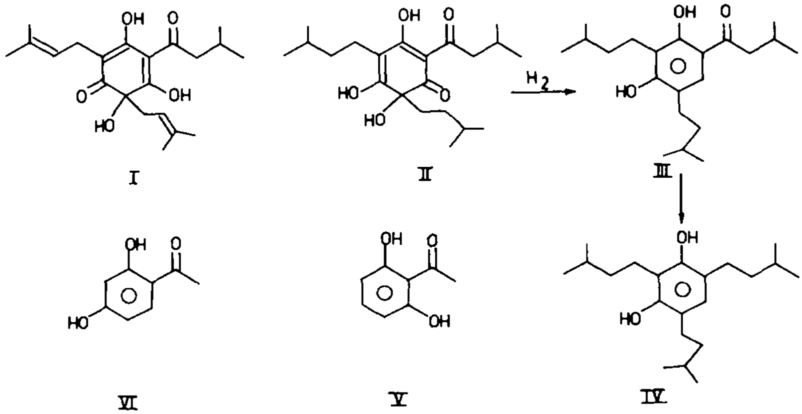
hydrohumulone only stops after ±5 moles of hydrogen are consumed. The reaction mixture was chromatographed on silicagel with benzene as eluting solvent. The crude product was crystallized from iso-octane giving white crystals, m.p. 70–70·5°. Spectral investigation, elemental analysis and comparison with reference compounds proved this to be 2,4-di-(3-methyl)-butyl-6-(3-methyl)-butanoylresorcinol (III). The UV spectrum in 0·1N HCl shows maxima at 237 and 287 mμ and in 0·1N NaOH at 236 and 357 mμ. The calculated UV maxima at longest wave length, following Scott's tables[7] for the 4-acyl derivative III are 284 mμ in acid and 341 mμ in alkaline medium; for the isomeric 2-acyl resorcinol derivative the calculations give 266 mμ in acid and 274 mμ in alkaline medium.

The IR spectrum shows bands at 3500–3100 cm^{-1} (OH), 1630 cm^{-1} (C=O), 1580 cm^{-1} (phenyl). The mol wt according to the mass spectrum is 334. The NMR spectrum is consistent with the proposed structure. It has a doublet at $\delta = 0{\cdot}99$ for the gem-dimethyl groups of the 3-methylbutyl side chains, another at $\delta = 1{\cdot}03$ for the gem-dimethyl groups of the 3-methylbutanoyl side chain. The coupling constants are 6 cps. The benzylic protons give a triplet around $\delta = 2{\cdot}52$, while a doublet is found at $\delta = 2{\cdot}72$ for the methylene protons next to the carbonyl function. The aromatic proton lies at $\delta = 7{\cdot}20$. Further, the non-bonded enolic hydroxylic function gives a signal at $\delta = 5{\cdot}2$, the strongly bonded enolic hydroxylic function at $\delta = 12{\cdot}94$.

With a large amount of very active hydrogenation catalyst, the same perhydrogenation procedure yielded 2,4,6-tri-(3-methyl)-butyl-resorcinol (IV) which is a white solid, m.p. 128–129°. This hydrogenolysis of the CO group of the 3-methyl-butanoyl side chain is normal for 5-membered ring hops derived compounds, but it is the first time that such a hydrogenolysis is reported in this field for a 6-membered ring compound. The structure of the 2,4,6-tri-(3-methyl)-butyl-resorcinol follows from its spectral properties. The UV spectrum in 0·1N HCl shows maxima at 234 and 286 mμ and in 0·1N NaOH at 232 and 355 mμ. The IR spectrum shows main bands at 3630 cm^{-1} (OH), 1380 cm^{-1} and 1200 cm^{-1}. The mol wt according to the mass spectrum is 320. The NMR spectrum is consistent with the proposed structure.

Chart 1



As the 2,4-di-(3-methyl)butyl-6-(3-methyl)butanoylresorcinol (III) is a key substance in this paper, its structure had to be proved unequivocally. Therefore 2-and

4-acetylresorcinol (V and VI) were synthesized as reference compounds and their NMR spectra compared with that of III.

1. *4-Acetylresorcinol* was synthesized according to Organic Syntheses.[8] It shows one NMR signal at δ:9·64 and another at δ:12·85, each integrating for one H atom. One enolic function is not intramolecularly H-bonded, while the other is strongly bonded.

2. *2-Acetylresorcinol* was synthesized following an unpublished procedure developed in our laboratory.[9] This shows a signal at δ:11·45, integrating for two H-atoms; therefore, both enolic OH functions are equivalent.

3. *2,4-Di-(3-methyl)butyl-6-(3-methyl)butanoylresorcinol.* As the reference compounds are only soluble in acetone, the NMR spectrum of the hydrogenolysis product was also recorded in acetone. In this solvent it shows a signal at δ:8·19 for a non-bonded enolic OH function and another at δ:13·28 for a strongly bonded enolic OH function.

In 4-acetylresorcinol only one of the two OH groups can form an intramolecular H-bond with the acetyl CO function. The two OH groups therefore give clearly different NMR signals with a large δ difference. When the acetyl group is located between the two OH groups of resorcinol (2-acetylresorcinol) the molecular symmetry and the rapid interconversion of both possible intramolecular H-bonded structures equalize the enolic protons. As expected in this case only one signal is produced with an intermediate δ value between the two values observed for 4-acetylresorcinol.

Since the hydrogenolysis product of (−)humulone produces two signals in the region to be expected the proposed structure (III) is confirmed.

Lead dioxide treatment of III in benzene produces an intense blue colour of the aroxyl radical. This is known to occur[10] with phenols when the *ortho* and *para* positions are substituted by bulky groups.

B. *Enolization of (−)humulone*

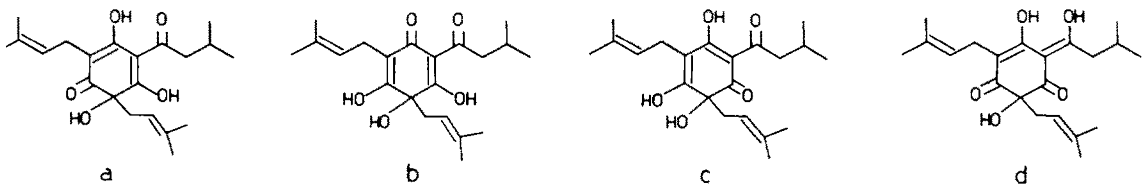
Active hydrogen determinations indicate that humulone probably occurs in a dienol form. This is confirmed by the NMR spectrum of very pure humulone (m.p. 72°—recrystallized several times from acetic acid). This spectrum recorded for a 10% carbon tetrachloride solution shows sharp peaks at δ:19·08 and δ:8·24 for one enolic function each and also a sharp peak at δ:5·15 for the tertiary alcohol function on the ring. With impure humulone this pattern is not observed as clearly. These facts exclude triketonic and monoenolic structures. The dienolic structure of (−) humulone can be formulated in different ways (chart II).

Structures *b* and *d* are impossible, as the strong Cotton-effect of humulone would not be possible on account of the symmetry around the chiral centre.

The remaining alternatives are structure *a*, which is the one usually employed for humulone, and *c*. The ring system of both possibilities has one CO and two enol functions. Structure *c* must be preferred over structure *a* from comparison of the humulone NMR spectrum with that of both acetylresorcinols I and II. Structure *a* would only show one enolic NMR signal integrating for two protons as in the case of 2-acetylresorcinol (see above).

During perhydrogenation of humulone, one of the oxygens of the ring keto-enol system is removed and it was assumed that this one differs from the other two. It is therefore logical that the carbonyl oxygen is hydrogenolysed while the two enol oxygens are left untouched. This is in contradiction with earlier work[11] showing that

CHART 2



hydrogenolysis of a CO function in simple ketones preferably occurs over the enol form. However, humulone is not a simple compound and the aromatization which occurs on removal of the OH function of the chiral centre probably plays a role in the reaction. Since the reaction product of this perhydrogenation is 2,4-di-(3-methyl) butyl-6-(3-methyl)butanoylresorcinol, the structure of humulone must be II in chart I, or *c* in chart II. An objection could be that the enol form of humulone is not the same in the acidic medium necessary for the hydrogenation, as in the normal state. Against this hypothesis is the important fact that the UV and NMR spectra of humulone in acidic or in neutral solution are identical.

C. *The absolute configuration of* (−)*humulone*

I. *By ORD.* The ORD curve of (−)humulone (Fig. I) shows a negative Cotton effect. The extrema are situated at $\lambda = 385$ mμ and $\lambda = 329$ mμ, while wavelengths where the optical rotation is zero, correspond to the absorption maxima in the UV region. The Cotton effect is caused by the asymmetry induced in the CO chromophore by the asymmetric environment of the chiral centre. Normally, the absorption band, owing to the $n—\pi^{x}$ transition in the CO chromophore is situated near 290 mμ. The higher value, here observed, is explained by the conjugation of the CO chromophore with two double bonds in α—β and γ—δ position. Moreover the pseudo axial OH group causes an additional red shift of 14–20 mμ. The humulone molecule is flexible at C atoms 3 and 4 as is readily seen on a Dreiding model and there are two important conformations, one with the tertiary OH group on the chiral centre in pseudo axial position and one in pseudo equatorial position. The octant diagrams presented in the usual way for the CO chromophore of the two forms in the two possible configurations are shown in chart III.

If we assume that the 3-methyl-2-butenyl side chain takes preferentially the pseudo-equatorial position, conformations (1) and (2′) are preferred.

The octant rule,[3] modified for unsaturated CO systems by Whalley[12] and checked by Snatzke,[13] predicts a negative Cotton-effect for conformation (2′) and a positive one for conformation (1). However, this helicity rule was criticized by Kuriyama *et al.*[14] These authors prove that, when the angle of skew is small in the unsaturated CO chromophore, the chirality contribution may be partially or completely offset by electronic interaction of the type observed in asymmetrically perturbed, intrinsically symmetric chromophores. The non validity of the rule applies especially in the presence of a hetero-atom homoconjugated with the unsaturated chromophore, such as in the present case. Here the effect will be most important, because the OH group bonded to the chiral centre, is situated next to the CO function. The exact solution of the problem can be found by a reasoning analogous to the octant rule.[3] Conformation

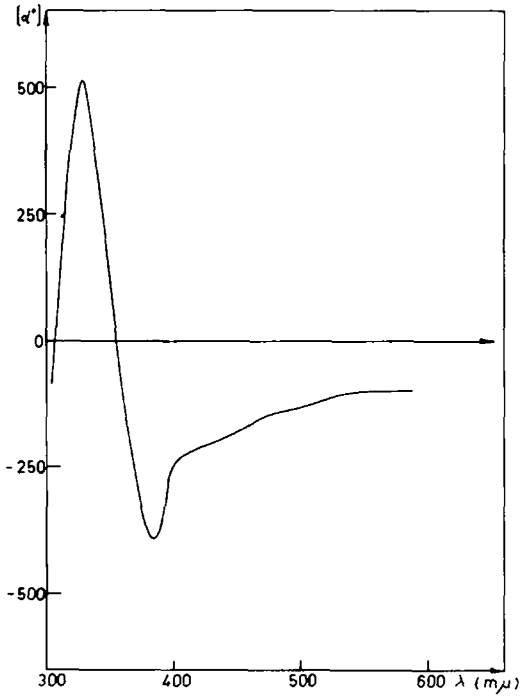
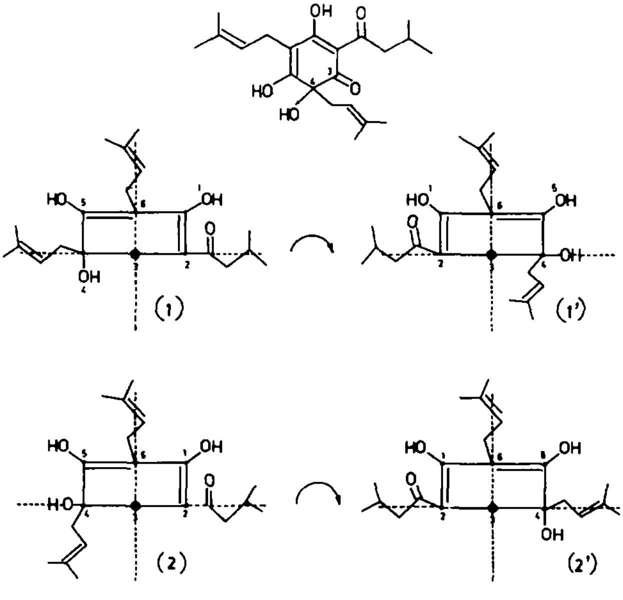

[α°]
500
250
0
-250
-500
300
400
500
600 λ (mμ)

FIG. 1

CHART 3


OH
O
HO
HO
3
4
HO
OH
OH
1
2
3
4
5
6
(1)
HO
OH
OH
O
1
2
3
4
5
6
(1')
HO
OH
HO
O
1
2
3
4
5
6
(2)
HO
OH
OH
O
1
2
3
4
5
6
(2')

(1) should give a negative Cotton-effect, conformation 2′) a positive one. The experimentally observed negative Cotton-effect is only compatible with conformation (1). Application of the Cahn-Ingold-Prelog sequence[15] rule leads to the *R* configuration. So we conclude that (−)-humulone has the *R* configuration.

II. *From the ozonolysis of* (−)*tetrahydrohumulone.* When the ozonide of (−)tetrahydrohumulone is decomposed with water, the major reaction products are 3-methyl-butanoic and 4-methyl-pentanoic acid. Cyclic byproducts of this ozonization are still under investigation. When the ozonide is decomposed with zinc dust and glacial acetic acid, the results are most interesting. The mixture obtained was worked up as described (Experimental) and treated with diazomethane and separated by preparative GC. A chromatogram is shown in Fig. II.

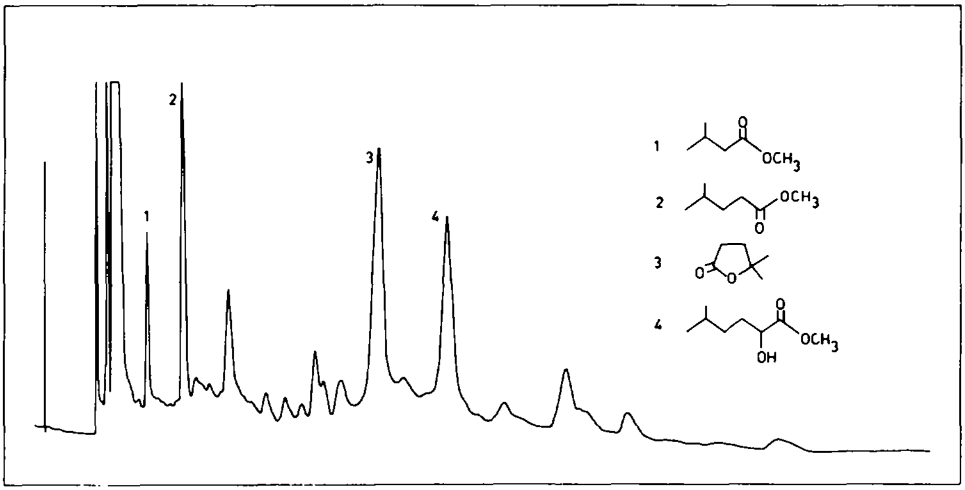


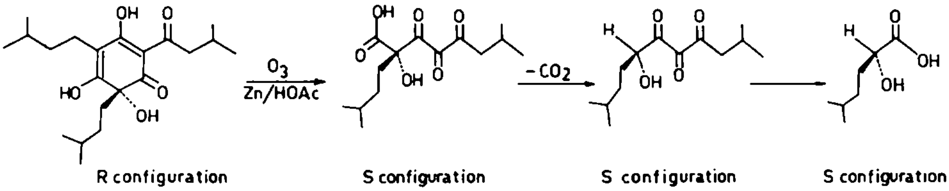
FIG. 2

Peaks 1 and 2 are again the methyl esters of 3-methyl-butanoic and 4-methyl-pentanoic acid but peak 4 proved to be optically active 2-hydroxy-5-methylhexanoic acid methyl ester. Surprisingly, this compound has not been described in the literature. Its structure was therefore carefully analysed. The mol wt as found by mass spectrometry is 160 and the MS and NMR spectrum are completely consistent with the advanced formulation. The following specific optical rotations were found for a 1% solution in methanol: λ 589 : [α] = 0·5°; λ 578 : [α] = 0·75°; λ 546 : [α] = 0·75°; λ 436 : [α] = 1·25°; λ 365 : [α] = 2·75°. The acid therefore gives a positive ORD plain curve. 2-Hydroxy-5-methyl-hexanoic acid was synthesized as follows. Diethylmalonate was alkylated with 3-methylbutyl bromide, the reaction product chlorinated at 80° and the 3-methylbutyl chloromalonate hydrolysed with potassium hydroxide. The 3-methylbutyl-hydroxymalonic acid was decarboxylated affording the desired 2-hydroxy-5-methylhexanoic acid.

The GC retention time of the methyl ester of the synthesized 2-hydroxy-5-methylhexanoic acid was compared with that of the ester isolated from the ozonolysis of tetrahydrohumulone, and proved to be the same. The IR, NMR and MS data of the synthetic and natural substances are also completely concordant. The absolute configuration of the 2-hydroxycarboxylic acids is known: the D series with *R*-configuration produce a negative ORD plain curve, while the L series with *S*-configuration give a positive one.[16]

The $[\alpha]_D^{20}$ value of pure optically active 2-hydroxy-5-methylhexanoic acid is not given in the literature. From the value for L-2-hydroxypropanoic acid (2·3°) L-2-hydroxyhexadecanoic acid (1·0°) D-2-hydroxy-3-methylbutanoic acid (–1·81°) and D-2-hydroxyheptanoic acid (–1·0°) it can be deduced that the optical purity of the L-2-hydroxy-5-methylhexanoic acid isolated is of the order of 30–50%. The chemical sequences for the formation of this acid can be formulated as follows in chart IV.

CHART 4



A hydroxydicarboxylic acid cannot be considered as intermediate in chart IV, since this would surely have led to an optically inactive product. Decarboxylation at the chiral centre therefore probably occurs before the polycarbonyl side chain is oxidized to a carboxyl function. Decarboxylation of asymmetric β-keto carboxylic acids can lead to racemization, but according to Cram, *et al.*,[17] Ferris and Miller[18] and also Finnegan and Knutson,[19] retention of configuration occurs very often.

Since the 2-hydroxy-5-methylhexanoic acid isolated by humulone ozonolysis is optically active and belongs to the L series, it is therefore most probable that (–)humulone is also an L form. In the Cahn-Ingold-Prelog nomenclature[12] the L-2-hydroxy-5-methylhexanoic acid is the *S* form. For (–)humulone however the sequence order of the substituents is such that by the same nomenclature it has the *R* configuration.

CONCLUSION

Interpretation of the ORD curve of (–)humulone leads to the *R* -configuration if we assume a preferential pseudo equatorial position for the bulkiest substituent on the chiral centre. This is a logical and usual supposition and even more probable in this case as the difference in mass units is large (69 – 17 = 52).

Ozonization experiments of (–)tetrahydrohumulone also lead to the *R* configuration for (–)humulone if we accept that the decarboxylation involved occurs with retention of the configuration.

The identical conclusion for two independent and plausible solutions for the hitherto unknown configuration of (–)humulone further supports the result obtained.

EXPERIMENTAL

Perhydrogenation of (–)tetrahydrohumulone

To 1 g tetrahydrohumulone dissolved in 20 ml MeOH, 5% PtO_2 (Adams' catalyst) and 2% Pt as chloroplatinic acid was added. After complete hydrogenation the solvent was evaporated and the residue chromatographed on silica gel with benzene as eluent. The extinction of the collected fractions was measured at $\lambda = 290$ mμ. The fractions giving an extinction were collected and the solvent evaporated. The residue was recrystallized from cold iso-octane giving white crystalline 2,4-di-(3-methyl)butyl-6-(3-methyl)-butanoyl resorcinol, m.p. 70–70·5° (yield 35–40%). With very active catalyst in large excess the same procedure yielded 2,4,6-tri-(3-methyl)butylresorcinol, m.p. 128–129°.

4-Acetylresorcinol. 81·7 g anhyd $ZnCl_2$ was heated with 81 g glacial AcOH. To the hot mixture, 55 g resorcinol was added. The soln was heated to boiling pt. Then, the flame was removed and the reaction allowed to complete itself. The soln was diluted with 150 ml of a mixture of 1 vol conc HCl and 1 vol water. The dark red soln was cooled to 5°. The resulting ppt was washed repeatedly with dil HClaq. It was distilled at 179–181° C/10 mm Hg (yield = 60%) yielding an orange product m.p. 142–145°.

2-Acetylresorcinol.[9] 77 g 2-acetyl-1,3-cyclohexanedione was boiled for 3 hr with one equiv N-chlorosuccinimide (67 g) in 200 ml CCl_4. The oily reaction mixture was distilled at 97°/0·03 mmHg. The distillate gave 76 g white crystalline (m.p. 41°) 2-acetyl-2-chloro-1,3-cyclohexanedione. This was boiled in 200 ml DMF containing 25% HCl and thus rearranged to 2-acetyl-4-chloro-1,3-cyclohexanedione which immediately lost HCl to form 2-acetylresorcinol. The solvent was evaporated, the residue extracted with ether and washed with sat $NaHCO_3$aq. After evaporating the ether, the residue solidified (yield 76%). Sublimation at 150°/0·03 mm Hg produced a yellow product, m.p. 156–157°.

Ozonolysis of (−)tetrahydrohumulone

In a typical experiment, 1 g tetrahydrohumulone, dissolved in 10 ml EtOAc was ozonized for several hr at −20°. The ozonide was decomposed with Zn dust/glacial AcOH as follows. 10 g Zn dust was suspended in 50 ml glacial AcOH. The ozonized soln was added dropwise to the suspension. Afterwards, the mixture was heated at 85°. The Zn was removed and the solvent evaporated. The residue was treated with an excess diazomethane. After removal of the solvents, the degradation products were separated by preparative GC.

The GC separations were carried out on a 20 m column (content 1·2 lit) with internal diameter 8 mm, filled with chromosorb W 30/60, coated with 25% polytetramethylene oxide (PTMO) as stationary phase. Hydrogen was used as mobile phase. The operating temp was 190°.

Preparation of 2-hydroxy-5-methylhexanoic acid

(a) *Preparation of 3-methylbutyldiethylmalonate.* 23 g Na was allowed to react with 500 ml "super-dry" EtOH. One equiv of diethylmalonate and one equiv of 3-methylbutyl bromide was added very slowly. The reaction mixture was refluxed for 2 hr. After addition of 500 ml water, the oily layer was separated and extracted twice with ether. The combined extracts were dried over Na_2SO_4. The ether was removed and the residue distilled. The portion, distilling at 115–125°/14 mm contained pure 3-methylbutyl diethylmalonate (yield 83%).

(b) *Chloro-3-methylbutyl diethylmalonate.* 50 g of 3-methylbutyl diethylmalonate was heated at 80° and chlorinated. The reaction proceeded quantitatively and only one Cl atom was introduced. The chlorinated product distilled at 140–141°/14 mm Hg.

(c) *2-Hydroxy-5-methylhexanoic acid.* 30 g chloro-3-methylbutyl diethylmalonate was treated with 20 ml water and 20 g KOH. The first reaction was very violent. After the solution had refluxed for 4 hr, the alcohol was distilled off. A slight excess of H_2SO_4 was added to the remaining soln, which was further refluxed for 4 hr.

The upper oily layer was separated and the aqueous layer extracted with ether. The combined extracts were dried over Na_2SO_4. The ether was removed and the residue distilled. The fraction distilling at 160–162°/15 mm Hg was pure 2-hydroxy-5-methylhexanoic acid which was recrystallized from iso-octane (m.p. 59–59·5°; (yield 53%).

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