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STRUCTURE OF EUCOMMIOL, A NEW CYCLOPENTENOID-TETROL FROM *EUCOMMIA ULMOIDES*[a]

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Abstract—Isolation of Eucommiol a new cyclopentenoid-tetrol from *Eucommia Ulmoides* (Eucommiaceae) is described. Its structure and absolute stereochemistry have been demonstrated.

Chromatographic examination of an ethanolic extract of *Eucommia Ulmoides* (Eucommiaceae) has revealed the presence of at least six compounds with probable iridoid structure (positive test with vanillin reagent[1]). After a purification,[1] the components were separated by repeated chromatography.

Four of them have been identified† as aucubin 1,[2] the main constituent, and harpagide acetate,[3] ajugoside[4] and reptoside[5] which are present in small amounts.

Another two new compounds (with $R_f = 0{\cdot}51$ (2) and 0·17 respectively, see Experimental) give an olive-brown colour with vanillin reagent and are present in the plant only in the autumn.

In the present paper we report the structure and absolute stereochemistry of the less polar compound 2, named Eucommiol.

Compound 2 is a hygroscopic and viscous colourless liquid, easily water-soluble with a neutral reaction: its molecular formula is $C_9H_{16}O_4$ and $[\alpha]_D^{25} - 30{\cdot}5$. The UV spectrum shows an absorption at λ_{max} 206 nm ($\log \epsilon = 3{\cdot}8$) indicative of an isolated C=C double bond. The total absence of any PMR peaks in the region downfield from the HDO signal excludes the presence in 2 of the enol-ether grouping characteristic of all iridoids. The weak IR band at 1665 cm^{-1} and the transparence of the 1000–670 cm^{-1} region (olefinic δ_{C-H} out of plane vibrations) are in agreement with the presence of a tetrasubstituted double bond in 2. The IR spectrum of 2 also shows a strong and broad OH absorption at 3340 cm^{-1}.

Under mild acetylation conditions, 2 yields the acetyl-derivative 3 which shows no OH absorption in the IR spectrum and contains (PMR) four acetyl groups.

The comparison of 60 MHz PMR spectra of 2 (D_2O) and 3 ($CDCl_3$) clarifies the nature of the four OH groups. The triplet (2H) at 3·71 δ shifts to 4·13δ ($\Delta\delta = 0{\cdot}42$ ppm) on acetylation: hence it has been assigned to the $-CH_2-$ of a $-CH_2\underline{CH_2}OH$ sequence. The complex multiplet (5H) centered at 4·23δ in the PMR spectrum of 2 turns—in the spectrum of 3—into a broad singlet (4H) at 4·72δ and a multiplet (1H) at 5·08δ, with a downfield shift of 0·50 and 0·85 ppm respectively. These spectroscopic data are in agreement with the presence of two equivalent allylic-CH_2OH groups and of one secondary OH group in 2.

Chemical support for the presence in 2 of primary allylic alcoholic functions is given, in absence of other oxidizable groups, by the positive reaction at room temperature with Tollen's reagent.[6]

The chemical and spectroscopic results above described suggest for Eucommiol structure 2

The proposed structure is well supported by the detailed analysis of the 100 MHz PMR spectrum (D_2O) of 2, greatly simplified by the use of double resonance technique (Fig 1).

The complex multiplet (24 lines) at 1·70–2·50δ has been assigned to the methylene $C_2^{\prime\prime}H_AH_B$, since double irradiation of the triplet (2H) at 4·11δ ($J = 7{\cdot}0$ Hz), assigned to the methylene protons on $C_2^{\prime\prime}$ simplifies the complex multiplet in two quartets constituting the AB part of an ABX system where $A = H_A$, $B = H_B$ and $X = H\text{–}C(2)$ ($\delta_A = 2{\cdot}31$, $\delta_B = 1{\cdot}92$, $\delta_X = 3{\cdot}15$; $J_{AB} = J_{gem} = 14{\cdot}0$ Hz, $J_{AX} = J_{gauche} = 3{\cdot}5$ Hz and $J_{BX} = J_{trans} = 9{\cdot}5$ Hz).‡ The complex pattern at 2·5–3·5δ (allylic region) has been assigned to the allylic protons on C(2) and C(5). In fact by double irradiation of the multiplet at 4·64δ, assigned to the two allylic $-CH_2OH$ groups and to the H–C(1) proton,§ the complex pattern turns into two systems: (1) a quartet at 3·15δ, assigned to the H–C(2) proton which is the X part of the ABX system previously described, as demonstrated by the identity of the coupling constants values of the quartet ($J_{AX} = 3{\cdot}5$ Hz and $J_{BX} = 9{\cdot}5$ Hz) with those involved in the AB part of the ABX system; (2) a simple two spin AB pattern, assigned to the geminal protons on C(5) ($\delta_1 = 3{\cdot}30$, $\delta_2 = 2{\cdot}77$, $J_{gem} = 18{\cdot}0$ Hz)[7] which is the A'B' part of another A'B'X' system where $A' = H_1\text{–}C(5)$, $B' = H_2\text{–}C(5)$ and $X' = H\text{–}C(1)$.

Chemical and further spectroscopic evidence support-

[a]The investigation were supported by the "C.N.R."

[b]In alphabetical order.

†The authors are greatly indebted to Prof. M. Guiso for providing these samples.

‡In this analysis δ and J values have been calculated from a first order treatment and result nearly identical with those found in the ABX calculations.

§The accidental coincidence of the chemical shift of these protons allows to have a triple resonance experiment with a simple irradiation.

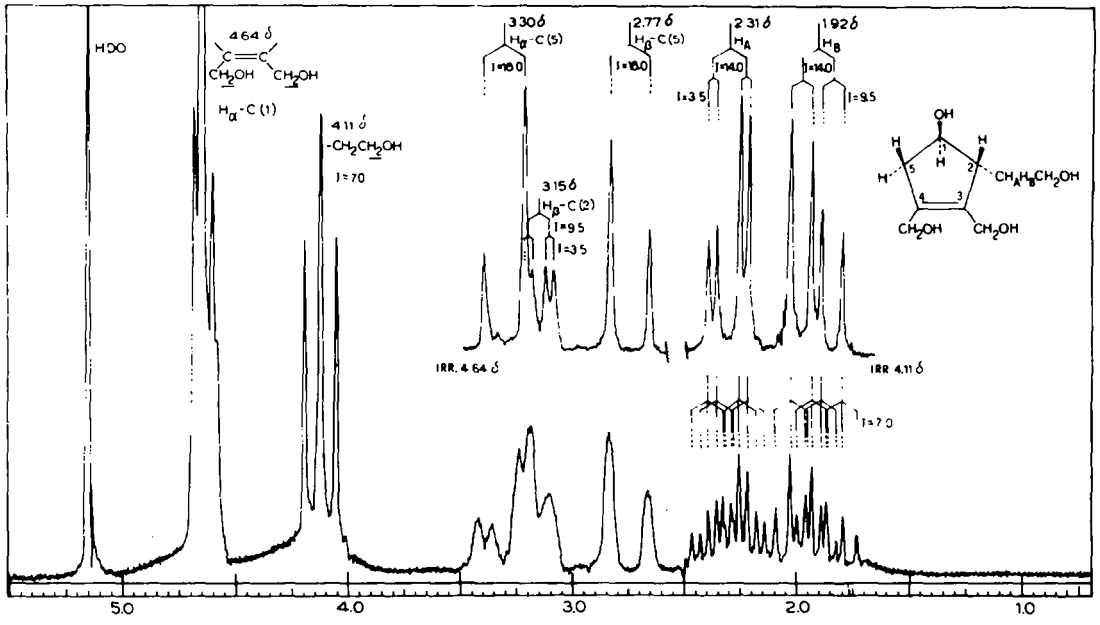
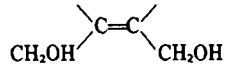


Fig 1. The single and double ^1H resonance spectra at 100 MHz of Eucommiol (2) in D_2O solution (TMS as external reference).

ing structure 2 and particularly the



moiety are given below.

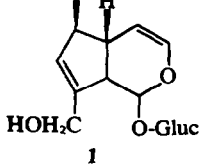
Eucommiol is converted, by Li/NH_3 reduction,[8] into a bisdeoxyderivative 4 whose PMR spectrum shows the presence of two vinylic methyls (broad singlet at 1·60δ, 6H) and the absence of allylic $-CH_2OH$ groups absorption. The evidence for the primary OH at C_2'' and of the secondary OH at C(1), has been obtained by acetylation of 4. In fact the PMR spectrum of the acetyl derivative 5 ($CDCl_3$) shows an acetyl peak at 2·03δ (6H) and in comparison with that of 4, the expected downfield shift of approximately 1 ppm for the multiplet due to the H–C(1) proton and of approximately 0·4 ppm for the triplet due to the protons on C_2''.

The presence in Eucommiol of two allylic $-CH_2OH$ groups on contiguous C(3) and C(4) sp^2 hybridised C atoms is confirmed by the exclusive and easily obtained mono-O-isopropylidene derivative 6 whose PMR spectrum shows the presence of one isopropylidene grouping (singlet at 1·43δ, 6H). Further acetylation of 6 affords the diacetyl derivative 7 whose PMR spectrum shows an acetyl peak at 2·04δ (6H). In comparison with the PMR spectrum of 6, the triplet assigned to the methylene protons on C_2'' is here displaced downfield by ~0·4 ppm (3·78δ → 4·12δ) while the multiplet assigned to the H-C(1) proton of ~1 ppm (4·16δ → 5·10δ). The complex signal of the two allylic $-\underline{CH_2}OH$ groups does not move ($\Delta\delta$ = 0·03 ppm) and is partially covered by the triplet of the acetoxyl bearing methylene protons ($-CH_2\underline{CH_2}OAc$).

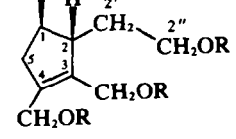
To establish the absolute stereochemistry of Eucommiol we have prepared, by selective tosylation of the primary OH group of 4, the mono-O-tosyl derivative 8 which has been used to determine with Horeau's method,[9] the absolute C(1) configuration. The secondary OH of 8 has been completely esterified by reaction with racemic α-phenylbutyric anhydride. The dextrorotatory *S*-α-phenylbutyric acid obtained (optical yield 11%) indicates the *R*-configuration of the asymmetric C(1) C atom. Thus in 2 H–C(1) has an α absolute configuration.

The single and decoupled ^1H resonance spectra of the tetra-O-acetyl eucommiol 3 (Fig 2) shows the relative stereochemistry of 2 and confirms its structure.

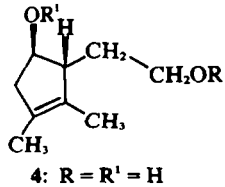
The accidental coincidence of the $H_α$–C(1) signals with



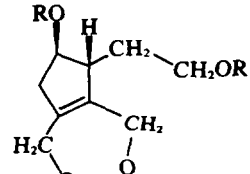
1



2: R = H
3: R = Ac



4: R = R' = H
5: R = R' = Ac
8: R = Ts, R' = H



6: R = H
7: R = Ac

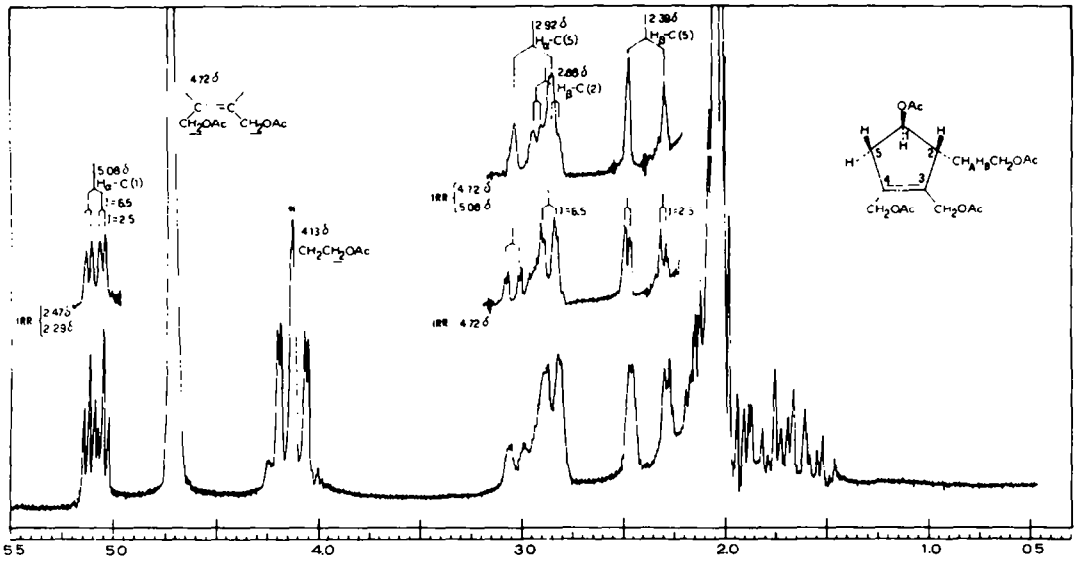


Fig 2. Single and multiple ^1H resonance spectra at 100 MHz of acetyl eucommiol (3) in $CDCl_3$ solution (TMS as internal reference).

that of the two allylic $-CH_2OH$ groups, found in the spectrum of 2, does not occur in the spectrum of 3 where the acetoxyl bearing methine proton absorbs at 5·08δ while the two allylic $-CH_2OAc$ groups at 4·72δ. Double irradiation of the latter signal sharpens the quartets at 2·92δ and 2·39δ (assigned to the geminal protons on C(5)) because of the collapse of the small splittings due to the homoallylic and long range coupling.[10]†

The low field quartet (2·92δ) has been assigned to the H_α–C(5) proton on the ground of the splitting value (J = 6·5 Hz) found for the coupling with H_α–C(1) proton that is characteristic of a *cis* configuration between the two protons ($J_{5\alpha,1\alpha} = J_{cis}$). In fact it is well known that in cyclopentene derivatives it is J *cis* > J *trans*.[11] The remaining quartet (2·39δ), with the coupling constant $J_{5\beta,1\alpha} = J_{trans}$ = 2·5 Hz,[11] has been obviously assigned to the H_β–C(5) proton. The multiplet relative to the H–C(2) proton overlaps the quartet at 2·92δ, assigned to the H_α–C(5) proton. Simultaneous irradiation with two r.f. fields (triple resonance) of allylic $-CH_2OAc$ groups at 4·72δ and of H_α–C(1) proton at 5·08δ, gives rise to the same decoupled pattern observed in the double resonance spectrum of 2 (Fig 1): the AB pattern, assigned to the geminal H_α–C(5) and H_β–C(5) protons, and the quartet at 2·88δ assigned to the H–C(2) proton. The H_α–C(1) proton at 5·08δ, which appears as a doublet of triplets,‡ is the X′ part of the A′B′X′ system (where A′ = H_α–C(5), B′ = H_β–C(5) and X′ = H_α–C(1)) and is further on coupled with the H–C(2) proton. By triple irradiation of the H_β–C(5) signals at 2·29 and 2·47δ,§ the doublet of triplets at 5·08δ turns into a quartet, because the H_α–C(1) proton remains coupled only with the H_α–C(5) proton (J = 6·5 Hz) and in addition with the proton on C(2) (J = 2·5 Hz). The value of 2·5 Hz, found for the coupling of the H_α–C(1) proton with the proton on C(2), establish[11] a "*trans*" configuration between the two protons and therefore a β absolute configuration for the proton on C(2).

Eucommiol is probably related to the aglycone of aucubin 1, which is always present in the plant as main iridoid constituent.

Work is in progress to establish a chemical correlation between these two compounds and to elucidate the structure of the more polar compound with R_f = 0·17.

EXPERIMENTAL

Whatman cellulose powder (standard grade) and Merck silica gel (140–230 mesh) were used for column chromatography. Silica gel was washed several times with hot water, acetone and afterwards dried *in vacuo* and activated at 120° for 8 h. Silica gel F_{254} and cellulose precoated plates were used in TLC. Chromatograms on paper (Schleicher & Schüll nr 2043 Mgl) were run in n-ButOH–AcOH–H_2O 63:10:27 and detected by spraying with a soln of vanillin (1 g) and conc HCl (3 ml) in MeOH (150 ml) and heating for 2–3 min at 100°. The PMR spectra were taken with Varian A-60 and HA-100 spectrometers by using $CDCl_3$ solns containing TMS as internal reference and D_2O solns containing TMS as external reference for the spectra at 100 MHz; HDO signal at 4·70δ was used as internal standard for those at 60 MHz. The PMDR and PMTR experiments were made by using Hewlett-Packard 4204 A and HP 200 CDR audio-oscillators in the TMS locked mode and frequency sweep operation. Chemical

*Each line of the triplet at 4·13δ is here splitted into a doublet probably because the internal rotation about the bond C H_AH_B–$\underline{C}H_2O$ is slowed down in the acetyl derivative 3.

†The smaller residual splittings are due to a long range coupling with one additional proton (probably H–C(2)).

‡The accidental identity of the coupling constant $J_{1\alpha,2\beta} = J_{1\alpha,5\beta}$ = 2·5 Hz, simplifies the expected theoric octet in a doublet of triplets.

§For this decoupling experiment, in which a large coupling constant is involved for the H_β–C(5) proton ($J_{5\alpha,5\beta}$ = 18·0 Hz), we have used the triple resonance because it is difficult to obtain sufficiently large r.f. field to effect complete decoupling.

shifts are expressed in δ values (ppm downfield from TMS) and J are quoted in Hz. IR spectra were determined on a Perkin-Elmer 257 instrument. UV spectra in EtOH on a Perkin-Elmer 137 instrument. Optical rotations were measured on a Perkin-Elmer 141 Polarimeter. Mass spectra were obtained with an AEI MS-12 at 70 eV, by using direct insertion at source temperature of 150°.

All described amorphous compounds, after chromatographic purification and before analytical determinations, were dissolved in water; the soln was filtered through Schleicher & Schüll "blauband" filter paper and dried *in vacuo* to constant weight (at 60°).

Isolation of iridoid fraction. Eucommia Ulmoides (4 Kg of fresh leaves collected in the autumn) was roughly chopped and twice extracted with EtOH (10 l. × 2) at room temp for 24 h. The combined EtOH solns were evaporated *in vacuo* (1·5 l.) to an aqueous suspension which was extracted 4 times with AcOEt (2 l.) and further concentrated *in vacuo* to 1·3 l. A chromatogram on paper revealed 6 spots with R_f 0·55 (pink lilac) reptoside, 0·48 (pink lilac) ajugoside, 0·37 (red lilac) harpagide acetate, 0·51 (olive-brown) eucommiol, 0·28 (pink-lilac) aucubin and 0·17 (olive-brown).

The final water soln was filtered through 0·4 Kg of decolorizing charcoal (Erba), suspended in water and stratified in gooch funnel (ϕ 13 cm). Mono and disaccharides were removed by elution with water (10 l), water-EtOH 95:5 (10 l) and water-EtOH 9:1 (11 l.). At this point positive reaction with vanillin reagent began. Compound with R_f 0·17, aucubin and eucommiol were eluted all together with water-EtOH 8:2 (25 l.) (fraction A); harpagide acetate, ajugoside and reptoside with water-EtOH 1:1) (14 l.) (fraction B).

Fraction A, evaporated *in vacuo*, gave 54·5 g of an amorphous residue. Fraction B was similarly treated to give 5·5 g of residue.

Eucommiol **2**. Fraction A (54·5 g) was chromatographed on silica gel (600 g). Elution with n-ButOH saturated with water gave in the first fraction eucommiol (9·5 g); afterwards a mixture of eucommiol and aucubin (0·7 g) and finally aucubin (12·5 g) and the compound with R_f 0·17 (15·5 g) as separated products. The crude eucommiol (0·8 g) was further chromatographed on cellulose powder (56 g). Elution with n-ButOH saturated with water gave pure eucommiol (0·6 g) as colourless viscous oil. For analytical purposes its purification was completed by distillation using a microsublimator (200°, 0·2 mmHg); $[\alpha]_D^{25}$ − 30·5 (MeOH, *c*. 1·08%). (Found: C, 57·16; H, 8·52. $C_9H_{16}O_4$ requires: C, 57·43; H, 8·59%).

MS (*m/e*, % of base peak): 170 (P–H_2O, 14·5), 152 (P-2 H_2O, 8·85), 139 (P–CH_2OH–H_2O, 88·5), 122 (17·7), 121 (16·5), 109 (20·5), 106 (20·5), 105 (17·7), 95 (29·5), 94 (17·7), 93 (100), 92 (17·7), 91 (69), 81 (26·5), 80 (41·2), 79 (68), 77 (50), 75 (17·7), 73 (23·5), 60 (41·2), 43 (17·7), 41 (68), 39 (31), 31 (35·3). H. R. measurements (RP ≥ 8000): *m/e* 170 ($C_9H_{14}O_3$), *m/e* 152 ($C_9H_{12}O_2$).

Tetra-O-acetyleucommiol **3**. To eucommiol (83 mg) dissolved in anhyd pyridine (0·5 ml) was added Ac_2O (1·0 ml). The soln was allowed to stand at room temp for 5 h, whereupon it was added MeOH (2 ml) at 0°. After 30′ on standing the soln was concentrated under reduced pression and Et_2O was added to the residue. The Et_2O soln was washed with cold dil HCl aq, water and dried (Na_2SO_4). The solvent was evaporated and the residue (105 mg) chromatographed on silica gel (10 g). Elution with benzene-Et_2O 7:3 gave **3** (80 mg) as colourless viscous oil. $[\alpha]_D^{25}$ − 20·7 (MeOH, *c*. 1·53%).

Bisdeoxyeucommiol **4** *and diacetate* **5**. To eucommiol (2 g), dissolved in absolute ethanol (6·5 ml), was added liquid ammonia (150 ml) and then, with stirring, lithium (0·74 g) in small pieces, over a period of 2 h (the temp was kept at −40°). The blue final soln was decolorized with abs EtOH (1 ml) and left overnight to allow the ammonia to evaporate. Removal of volatile liquids was completed at reduced pression. The solid residue was dissolved in water (100 ml) and extracted several times with AcOEt. The resulting soln, dried on Na_2SO_4, was evaporated *in vacuo*. The residue (330 mg) was chromatographed on silica gel (14 g). Elution with AcOEt-benzene 7:3 gave **4** (55 mg) as colourless viscous oil. (Found: C, 68·98; H, 10·35. $C_9H_{16}O_2$ requires: C, 69·19; H, 10·32%).

Acetylation of **4**, carried out as for **3**, gave pure **5** as colourless viscous oil.

Isopropylideneucommiol **6** *and diacetate* **7**. To eucommiol (100 mg) dissolved in 1 ml dry acetone, was added 1·2 ml of an acetone soln of $SnCl_2$ (500 mg in 3 ml). The soln was left at room temp for 1 h and then neutralized with sat $NaHCO_3$ aq. The suspension was centrifuged and the solid residue washed twice with acetone-water 1:1. The combined supernatant liquids were evaporated and the residue treated with abs EtOH. The filtered ethanolic soln. was evaporated and the residue (80 mg) chromatographed on silica gel (2·5 g). Elution with AcOEt-benzene 8:2 gave **6** (30 mg) as colourless viscous oil. (Found: C, 63·02; H, 8·57. $C_{12}H_{20}O_4$ requires: C, 63·14; H, 8·83%).

Acetylation of **6**, carried out as described for **3**, gave pure **7** as colourless viscous oil.

Tosylderivative **8**. To bisdeoxyeucommiol **6** (55 mg, 0·352 mmoles) dissolved in anhyd pyridine (2 ml) was added the *p*-toluensulphochloride (73 mg, 0·352 mmoles). The soln was stored at 0° overnight, then water (1 ml) was added. After 30′ stirring, Et_2O was added and the Et_2O soln washed with cold sat $NaHCO_3$ aq, cold 1N H_2SO_4, water and then dried (Na_2SO_4). The Et_2O soln was evaporated and the residue (70 mg) chromatographed on silica gel (3 g). Elution with AcOEt-benzene 8:2 gave **8** (22 mg). (Found: C, 62·15; H, 7·17; S, 10·09. $C_{16}H_{22}O_4S$ requires: C, 61·94; H, 7·10; S, 10·32%). PMR spectrum ($CDCl_3$, 60 MHz): 6·98–7·68δ (AA'BB', 4 arom. protons), 4·01δ (triplet, J = 7·0 Hz, $-CH_2\underline{CH_2}OTs$), 3·86δ (complex multiplet, $\rangle\underline{CH}OH$), 2·36δ (singlet, $Ph\underline{CH_3}$), 1·78δ (singlet, $\rangle CHO\underline{H}$), 1·51δ (broad singlet, $\underline{CH_3}\diagdown\!\!\diagup C{=}C\diagdown\!\!\diagup\underline{CH_3}$).

Absolute configuration of C(1)-(*Horeau's method*). To tosyl derivative **8** (50 mg) was added anhyd pyridine.(77 mg) and freshly distilled racemic α-phenylbutyric anhydride (160 mg). The mixture was left at room temp until complete esterification (2 h) and then diluted with cold Et_2O. The Et_2O soln was extracted twice at 0° with sat $NaHCO_3$ aq, the alkaline extracts were acidified at 0° and extracted 4 times with Et_2O. After drying on Na_2SO_4 and removal of the solvent at low temp., a residue of 28·0 mg (calcd. 26·5) of α-phenylbutyric acid was obtained. α = +0·30 (benzene, *c* = 1%); α calcd. = +1·8, optical yield 16%.

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