DETERMINATION OF THE ABSOLUTE CONFIGURATION OF SECONDARY ALCOHOLS BY MODIFIED HOREAU'S METHOD USING HPLC

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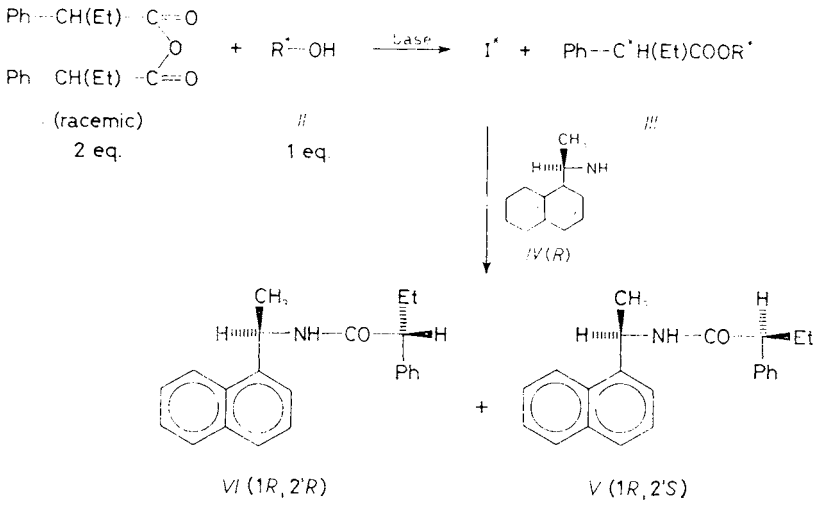
A method for determination of absolute configuration of secondary alcohols, based on a modified Horeau's method, has been developed. The ratio of (1*R*,2'*S*)- and (1*R*,2'*R*)-N-[1-(1-naphthyl)-ethyl]-2-phenylbutanamides (*V* and *VI*, respectively) was determined by HPLC on a straight phase. The method was tested on a series of steroid and terpene model compounds and was used in the determination of absolute configuration of 15-ripperten-3α-ol (*XV*), the defense substance of *Nasutitermes nigriceps* termites. The sensibility of the determination is 100 nmol of the alcohol.

The biological activity of natural as well as synthetic compounds is closely connected with their spatial structure. Knowing the spatial structure, and thus also the absolute configuration, one can assess the interaction of chemical compounds with receptors on the molecular level. Neither the known methods[1] used for determination of absolute configuration (X-ray diffraction, CD and ORD spectroscopy, NMR spectroscopy or biological test) nor the classical method of Horeau[2] has met our requirements concerning sensitivity, a certain universality and accessibility. We therefore modified the Horeau's method used for determination of absolute configuration of secondary alcohols[2]. The method is based on the reaction of a chiral alcohol with racemic 2-phenylbutanoic anhydride (*I*). The chiral alcohol reacts preferentially, according to the known mechanism, with one enantiomer of the anhydride and therefore, after the end of the reaction, the remaining excess anhydride is no more racemic. From the sign of optical rotation of the reaction mixture one can derive which of the enantiomers reacted preferentially and thus what is the absolute configuration of the studied alcohol. This method has been adapted for gas-liquid chromatography[3], its sensitivity being 1 μmol of the alcohol. Using the mentioned modification (Scheme 1) we determined the ratio of amides, *V*/*VI*, by HPLC. The high molar absorption coefficient of the amides *V* and *VI* in the UV spectrum enhanced the sensitivity up to 100 nmol of the alcohol.

The amides *V* and *VI* have very advantageous chromatographic properties[4]. Although they are diastereoisomers, they are separated already by TLC on silica gel G in hexane–ethyl acetate (5 : 1) (R_F of *V*: 0·44, of *VI*: 0·34). They retain their advantageous properties on HPLC on a straight phase (Table I, Fig. 1). Since in



SCHEME 1

hexane–2-propanol (96 : 4) the amine *IV* does not separate completely, it is better to use hexane–ethyl acetate (90 : 10). In this mobile phase both the amides *V* and *VI* and the unreacted amine *IV* separated well. An attempted separation of the amides on a reversed phase (C-18) in methanol–water (70 : 30) failed.

For the determination of absolute configuration by the discussed method the estimation of the amide ratio *V* : *VI* is important. We therefore checked the accuracy of the integration and reproducibility which in the described arrangement was better than ±0·6%. The sensitivity of the HPLC analysis was determined by the multilevel calibration method using diluted stock solutions. The achieved sensitivity (at 281 nm and signal : noise ratio > 100 : 1) was 1 nmol of the amides determined. To compare physical properties, the amides *VII* and *VIII* (configuration 1*S*,2'*R* and 1*S*,2'*S*, respectively) were prepared by reaction of racemic 2-phenylbutanoic anhydride (*I*) with (*S*)-(−)-1-(1-naphthyl)ethylamine.

The reaction of an optically active alcohol of the general formula *II* with 2 equivalents of racemic 2-phenylbutanoic anhydride (*I*) in pyridine affords preferentially one of the diastereoisomeric esters *III*. Since the anhydride is not racemized during the reaction[5], the remaining 1 equivalent will be optically active. This anhydride is

then reacted with (*R*)-(+)-1-(1-naphthyl)ethylamine (*IV*) to give a mixture of diastereoisomeric amides *V* and *VI*. The steric preference in this method is well known and the original methods[2,3] check its validity by model compounds of known absolute configuration, without assigning the individual chromatographic peaks to the amides *V* and *VI* (ref.[3]). Independently, we converted the partially optically active (*R*)-(−)-2-phenylbutanoic acid of known[6] absolute configuration into a mixture of amides *V* and *VI* in which the amide *VI* (with longer retention time) predominated (Table I). This means that the amide *V* (1*R*,2′*S*) is eluted first and its isomer *VI* (1*R*,2′*R*) as the second. This assignment corresponds to that found by Helmchen[7].

TABLE I

Chromatographic properties of compounds *IV*, *V* and *VI*

Mobile phase[a]	Compound	r_t, min	k'_i	$\alpha(i,j)$	$R(i,j)$
A	*VI*	15·12	4·03	$\alpha(V, VI)$ 1·78	$R(V, VI)$ 4·13
A	*V*	9·85	2·27	$\alpha(IV, V)$ 1·19	$R(IV, V)$ 4·72
A	*IV*	11·14	2·71		
B	*VI*	9·40	2·13	$\alpha(V, VI)$ 1·32	$R(V, VI)$ 2·62
B	*V*	7·85	1·61	$\alpha(IV, V)$ 1·14	$R(IV, V)$ 6·47
B	*IV*	7·24	1·41		

[a] Mobile phase A: hexane–ethyl acetate (90 : 10), mobile phase B: hexane–2-propanol (96 : 4).

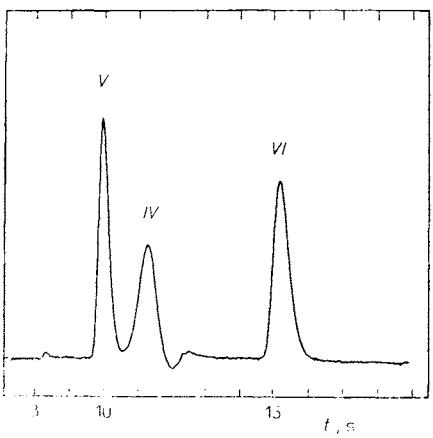
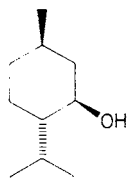


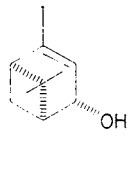
FIG. 1

Chromatography of compounds *IV*, *V* and *VI*

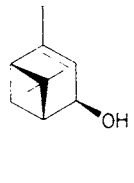
The results of absolute configuration determinations for secondary alcohols are given in Table II. The obtained diastereoisomeric excesses (d.e.) are relatively low, maximum 22%. To obtain a higher dependability of the determination, it is necessary to carry out at least two concurrent reactions and in each series of experiments with new stock solutions the model reaction should be repeated (in our case with cyclohexanol (*IX*) and (*R*)-(−)-menthol (*X*)). Since the reaction requires a long reaction time (24 h), we tried to enhance the reaction rate by addition of catalytic amounts of 4-dimethylaminopyridine. Although the acceleration did occur, the method led invariably to absolute configurations opposite to those derived by method *I*. We therefore assume that in the presence of 4-dimethylaminopyridine the ester *III* arises by another reaction course.



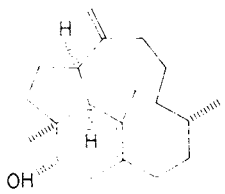
X



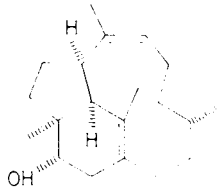
XI



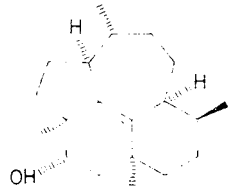
XII



XIII

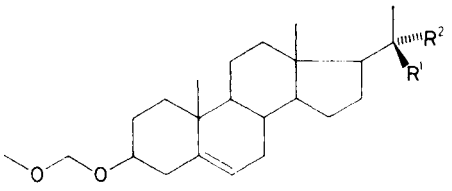


XIV

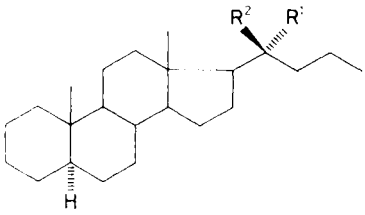


XV

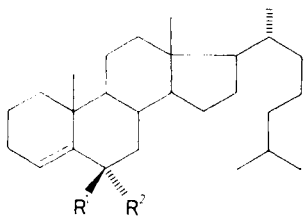
The applicability and dependability of the described method were checked with a series of steroid and terpene alcohols (Table II). We also studied the diterpenes *XIII* and *XIV* from *Nasutitermes nigriceps* termites the absolute configuration of which we had determined by the NMR spectroscopy[8]. For one of the compounds isolated by us previously — 15-ripperten-3α-ol (*XV*) — the absolute configuration was not known. Using the described method, we assigned its absolute configuration as 3*S*. Since the relative configuration of *XV* is already known from the NMR spectra and X-ray diffraction[9], its configuration is 1*S*, 3*S*, 4*S*, 7*S*, 8*R*, 11*S*, 12*S*.



XVI, $R^1 + R^2 = O$
XVII, $R^1 = H$; $R^2 = OH$
XVIII, $R^1 = OH$; $R^2 = H$



XIX, $R^1 = H$; $R^2 = OH$
XX, $R^1 = OH$; $R^2 = H$



XXI, $R^1 = H$; $R^2 = OH$
XXII, $R^1 = OH$; $R^2 = H$

EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Infrared spectra were taken on UR-20 (Zeiss, Jena) and Perkin–Elmer PE 580 spectrometers (wavenumbers in cm^{-1}), UV spectra were obtained with a Cary 219 (Varian) spectrometer in ethanol (cell thickness 1 cm, wavelengths in nm, molar absorption coefficients ε in mol m^{-2}). ^1H NMR spectra were measured on a Varian XL 200 (200 MHz) instrument in deuteriochloroform with tetramethylsilane as internal standard. All the parameters were obtained by first order analysis, chemical shifts are given in ppm (δ-scale) and coupling constants (J) in Hz. The reactions were monitored by TLC on silica gel G according to Stahl (Merck).

HPLC *analyses*. The determinations were carried out on an HP 1090 (Hewlett–Packard) instrument equipped with a DAD UV detector. Integrations were performed on an 8-channel DPU integrator and the chromatography was controlled with a Hewlett–Packard 85-B computer equipped with B-2 570 software (1984). The analyses were carried out on a steel column HP (4·2 × 250 mm) packed with SIL 100 straight phase (particle size 5 μm). Hexane–ethyl acetate (90 : 10; phase A, detection at 281 nm) or hexane–2-propanol (96 : 4; phase B, detection at 224 nm) were used as mobile phases; flow rate 1 ml/min.

Solvents and reagents. The solvents used in the microreactions were kept over molecular sieves 3A and distilled from calcium hydride under argon immediately before the reaction. Stock solutions of the reagents were stored in 100 μl vials with septum (Alfa-Ventron) and the liquids were transferred using Hamilton syringes (100 μl and 5 μl). Racemic 2-phenylbutanoic anhydride, (*R*)-(+)- and (*S*)-(−)-1-(1-naphtyl)ethylamines were commercial products (Fluka). The micro-

reactions were performed in freshly prepared glass capillaries (1 mm i.d.), solutions were filtered using a glass bubble pipette with a short column of Florisil (Fig. 2). Partially optically active (*R*)-(−)-2-phenylbutanoic acid was prepared according to the literature[6].

(1*R*,2'*S*)-N-[1-(1-Naphthyl)ethyl]-2-phenylbutanamide (*V*) and
(1*R*,2'*R*)-N-[1-(1-Naphthyl)ethyl]-2-phenylbutanamide (*VI*)

Racemic 2-phenylbutanoic anhydride (*I*, 144 mg; 0·46 mmol) and 4-dimethylaminopyridine (72 mg; 0·60 mmol) were dissolved in dry dichloromethane (1 ml) in a 10 ml flask under argon. After cooling of the solution with ice-cold water, a solution of (*R*)-(+)-1-(1-naphthyl)ethylamine (*IV*; 34 mg; 0·20 mmol) in dichloromethane (0·2 ml) was added through the septum by means of a syringe. After standing for 1·5 h in an ice bath, the reaction was quenched by pouring into ice-cold 1M-HCl (10 ml). Dichloromethane (10 ml) was added, the aqueous layer was separated

TABLE II

Absolute configuration of alcohols determined by method *I*

Compound	Absolute configuration (known)	Proportion of amides *V*(1*R*,2'*S*)/*VI*(1*R*,2'*R*)	Absolute configuration (found)
IX	achiral	52/48	achiral
X	*R*	68/32	*R*
XI	*S*	41/59	*S*
XII	*R*	55/45	*R*
XIII[a]	*S*	42/58	*S*
XIV[a]	*S*	43/57	*S*
XV[b]	unknown	45/55	*S*
XVII[c]	*S*	49/51	*S*
XVIII[c]	*R*	53/47	*R*
XIX[d]	*S*	48/52	*S*
XX[d]	*R*	53/47	*R*
XXI[e]	*S*	47/53	*S*
XXII[e]	*R*	53/47	*R*

[a] Ref.[8]; [b] ref.[9]; [c] configuration on $C_{(20)}$ determined from the shift of H-21 signal in NMR spectrum according to ref.[13]; [d] ref.[11]; [e] ref.[12].

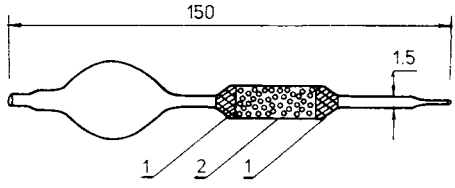


FIG. 2

Bubble pipette for filtration of the reaction mixture prior to the HPLC analysis (1 glass wool, 2 Florisil 100—200 mesh, 0·5 g). Dimensions given in mm

and the organic one was washed successively with water, saturated sodium hydrogen carbonate solution and water. After drying over anhydrous sodium sulfate and evaporation of the solvent in vacuo, the residue was repeatedly chromatographed on a column of silica gel (4 g) in hexane–ethyl acetate (4 : 1) to give 18·3 mg (29%) of the less polar amide *V* and 24·2 mg (38%) of the more polar amide *VI*.

Amide V: m.p. 155–156°C (hexane); $[\alpha]_D$ +57° (*c* 0·37, ethanol). UV spectrum, $\lambda_{max}(\varepsilon)$: 224·5 (7 970), 271 (659), 281 (792), 288 (536), 292 (548), 312·5 (37). IR spectrum ($CHCl_3$): 3 440 (NH), 1 670 (C=O), 1 602, 1 516, 708 (arom. ring). ^{1}H NMR spectrum: 0·84 t, 3 H (H-4′, J(3′, 4′) = 7·4); 1·53 d, 3 H (H-2, J(1, 2) = 6·6); 1·77 m, 1 H and 2·18 m, 1 H (2 × H-3′); 3·14 t, 1 H (H-2′, J(2′, 3′) = 7·5); 5·61 bd, 1 H (NH, J(NH, 1) = 8·5); 5·90 dq, 1 H (H-1, J(1, 2) = 6·6, J(1, NH) = 8·5); 7·24–7·57 m, 7·74–7·90 m and 8·05–8·12 m, 12 H (arom. H).

Amide VI: m.p. 152–153°C (hexane); $[\alpha]_D$ −20° (*c* 0·47, ethanol). UV spectrum, $\lambda_{max}(\varepsilon)$: 224 (7 486), 271 (627), 281 (718). IR spectrum ($CHCl_3$): 3 440 (NH), 1 668 (C=O), 1 599, 1 503 (arom. ring). ^{1}H NMR spectrum: 0·90 t, 3 H (H-4′, J(3′, 4′) = 7·4); 1·61 d, 3 H (H-2, J(1, 2) = = 6·7); 1·78 m, 1 H and 2·20 m, 1 H (2 × H-3′); 3·21 t, 1 H (H-2′, J(2′, 3′) = 7·6); 5·66 bd, 1 H (NH, J(NH, 1) = 7·0); 5·84 m, 1 H (H-1); 7·18–7·47 m and 7·70–7·89 m, 12 H (arom. H). For $C_{22}H_{23}NO$ (317·4) calculated: 83·24% C, 7·30% H, 4·41% N; found: 83·18% C, 7·18% H, 4·40% N.

(1*S*, 2′*R*)-N-[1-(1-Naphthyl)ethyl]-2-phenylbutanamide (*VII*) and
(1*S*, 2′*S*)-N-[1-(1-Naphthyl)ethyl]-2-phenylbutanamide (*VIII*)

(*S*)-(−)-1-(1-Naphthyl)ethylamine (34 mg) was converted into amide *VII* (18·2 mg; 29%), m.p. 155–157°C (hexane), $[\alpha]_D$ −51° (*c* 0·20, ethanol) and amide *VIII* (24·1 mg; 38%), m.p. 151–153°C (hexane), $[\alpha]_D$ +18° (*c* 0·17, ethanol) as described in the preceding experiment.

(1*R*, 2′*R*)-N-[1-(1-Naphthyl)ethyl]-2-phenylbutanamide (*VI*)

(*R*)-(−)-2-Phenylbutanoic acid (130 mg; 80% optical purity) in thionyl chloride (distilled; 1 ml) was heated to 60°C for 3 h. The excess thionyl chloride was distilled off, the remaining crude chloride was cooled with ice and mixed with a solution of 4-dimethylaminopyridine (50 mg) in dichloromethane (1 ml), followed by (*R*)-(+)-1-(1-naphthyl)ethylamine (*IV*; 115 mg) in dichloromethane (1 ml). The reaction mixture was immediately worked up by pouring on a small column of silica gel (1 g) and eluting the product with light petroleum–ether (7 : 3). The crude product (120 mg; 48%) contained 84% of the amide *VI* (longer retention time). Chromatography on a column of silica gel (5 g) in light petroleum–ether (5 : 1) afforded 6·1 mg of amide *V* and 13·5 mg of amide *VI*, in addition to an intermediate fraction.

(20*S*)-3β-Methoxymethoxy-5-pregnen-20-ol (*XVII*) and
(20*R*)-3β-Methoxymethoxy-5-pregnen-20-ol (*XVIII*)

Sodium (3 g) was added in small pieces at 80°C to a stirred solution of ketone *XVI* (ref.[10]; 470 mg) in a mixture of benzene (30 ml) and 1-propanol (45 ml). The mixture was refluxed for 1 h, cooled and partitioned between ether and water. The ethereal extract was washed with hydrochloric acid (1 : 4), saturated sodium hydrogen carbonate solution and water, dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo. The crude product was chromatographed on four preparative plates (200 × 200 × 0·7 mm) in benzene–ether (7 : 3) to give 122 mg of alcohol *XVII* and 81 mg of alcohol *XVIII*.

Alcohol XVII: m.p. 130–132°C (ether–light petroleum), $[\alpha]_D$ −54° (*c* 0·28, chloroform). IR spectrum ($CHCl_3$): 3 620, 3 470 (OH), 1 690 (C=C), 1 149, 1 105, 1 036, 915 (CH_3OCH_2O). ^1H NMR spectrum: 0·677 s, 3 H (3 × H-18); 1·007 s, 3 H (3 × H-19); 1·22 d, 3 H (3 × H-21, $J(20, 21) = 6·1$); 3·35 s, 3 H (OCH_3); 4·69 s, 2 H (OCH_2O); 5·36 bd, 1 H (H-6, $J = 4·5$). For $C_{23}H_{38}O_3$ (362·6) calculated: 76·20% C, 10·56% H; found: 75·93% C, 10·55% H.

Alcohol XVIII: m.p. 141–143°C (ether–light petroleum), $[\alpha]_D$ −72° (*c* 0·27, chloroform). IR spectrum ($CHCl_3$): 3 610, 3 490 (OH), 1 670 (C=C), 1 150, 1 105, 1 039, 915 (CH_3OCH_2O). ^1H NMR spectrum: 0·757 s, 3 H (3 × H-18); 1·007 s, 3 H (3 × H-19); 1·123 d, 3 H (3 × H-21, $J(20, 21) = 6·2$); 3·35 s, 3 H (OCH_3); 4·68 s, 2 H (OCH_2O); 5·35 d, 1 H (H-6, $J = 4·5$). For $C_{23}H_{38}O_3$ (362·6) calculated: 76·20% C, 10·56% H; found: 75·98% C, 10·60% H.

Execution of the Microreactions – General Procedure

The reactions were performed with 0·1–0·5 μmol of the studied alcohol. Under argon, the following amounts of the reactants were injected via syringe into the reaction capillary: method *1*: 2 equivalents of racemic anhydride *I* (0·2M solution in toluene) and 1 equivalent of the studied alcohol (0·1M solution in pyridine); method *2*: 2 equivalents of anhydride *I* (0·2M solution in toluene), 0·25 equivalent of 4-dimethylaminopyridine (0·1M solution in toluene) and 1 equivalent of the studied alcohol (0·1M solution in pyridine). The capillary was sealed and centrifuged. The reaction time was 24 h and 1·5 h for method *1* and *2*, respectively. The capillary was opened, 6 equivalents of (*R*)-(+)-amine *IV* (0·2M solution in toluene) were added and the capillary was sealed again. After 30 min 4·7M solution of perchloric acid in acetonitrile (50 μl) was added. The opened capillary in a 2 ml test tube was washed with ether–light petroleum (1 : 2; 100 μl) and the solution was filtered through a filtration device (Fig. 2). The filtrate was transferred into a 100 μl vial, concentrated under a stream of carbon dioxide and diluted with the mobile phase (30 μl) (Table I). The HPLC analysis was performed with 10 μl of this solution.

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