PERGAMON

Phytochemistry 58 (2001) 799–810

PHYTOCHEMISTRY

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# Chemical study of the essential oil of *Cyperus rotundus*

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Received 4 April 2001; received in revised form 25 June 2001

**Abstract**

Minor constituents of the essential oil of *Cyperus rotundus* have been investigated. The three new sesquiterpene hydrocarbons (−)-isorotundene, (−)-cypera-2,4(15)-diene, (−)-norrotundene and the ketone (+)-cyperadione were isolated and their structures elucidated. The absolute configuration of (−)-rotundene was derived by chemical correlation and enantioselective gas chromatography. 

*Keywords:* *Cyperus rotundus*; Cyperaceae; Sesquiterpenes; Norsesquiterpene; (−)-Isorotundene; (−)-Cypera-2,4(15)-diene; (−)-Norrotundene; (+)-Cyperadione

## 1. Introduction

*Cyperus rotundus* is a cosmopolitan sedge belonging to the family of the Cyperaceae. It is encountered in tropical, subtropical and temperate regions. *C. rotundus* is a traditional medicinal plant appearing among Indian, Chinese and Japanese natural drugs used against spasms and stomach disorders (Dassanayake and Fosberg, 1985). The essential oil as well as solvent extracts of the rhizomes of *C. rotundus* have been subject to numerous studies (Ohira et al., 1998 and cited literature) resulting in the isolation of many terpenoids. Here we describe the isolation and structure elucidation of new patchoulane and rotundane sesquiterpenes from the essential oil of this plant.

## 2. Results and discussion

The analysis of the essential oil of *Cyperus rotundus* by GC and GC–MS allowed the identification of cyprotene (**1**), cypera-2,4-diene (**2**), α-copaene (**3**), cyperene (**4**), α-selinene (**5**), rotundene (**6**), valencene (**7**), ylanga-2,4-diene (**8**), γ-gurjunene (**9**), *trans*-calamenene (**10**), δ-cadinene (**11**), γ-calacorene (**12**), *epi*-α-selinene (**13**), α-muurolene (**14**), γ-muurolene (**15**), cadalene (**16**), nootkatene (**17**) by comparison with a spectral library established under identical experimental conditions (Joulain and König, 1998), cyperotundone (**18**) (Hikino et al., 1966), mustakone (**19**) (Nyasse et al., 1988), cyperol (**20**) (Hikino et al., 1967), isocyperol (**21**) (Hikino et al., 1967) and α-cyperone (**22**) (Howe and McQuillin, 1955; Haaksma et al., 1992) (Fig. 1). However, several minor compounds could not be identified by their mass spectra and had to be isolated for further investigations. Consequently, the essential oil was first separated by silica chromatography into a hydrocarbon fraction and a more polar oxygenated fraction. The hydrocarbon fraction was further separated by column chromatography, TLC over silver nitrate precoated silica and preparative GC. This resulted in the isolation and characterization of isorotundene (**23**), cypera-2,4(15)-diene (**24**) and norrotundene (**25**). From the oxygenated fraction cyperadione (**26**), a patchoulane type sesquiterpene, was isolated.

### 2.1. (−)-Isorotundene (*23*)

The unknown compound **23** was assigned the molecular formula $C_{15}H_{24}$ ($[M]^+$ at $m/z = 204$). The $^{13}C$ NMR spectrum showed 15 carbon signals, two of which were olefinic ($\delta$ 107.9 and 151.08). Through the DEPT technique two methyl groups, seven methylene groups, four methine and two quaternary carbon atoms were identified. The compound has four unsaturations and only one double bond, therefore the presence of three rings

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PII: S0031-9422(01)00301-6

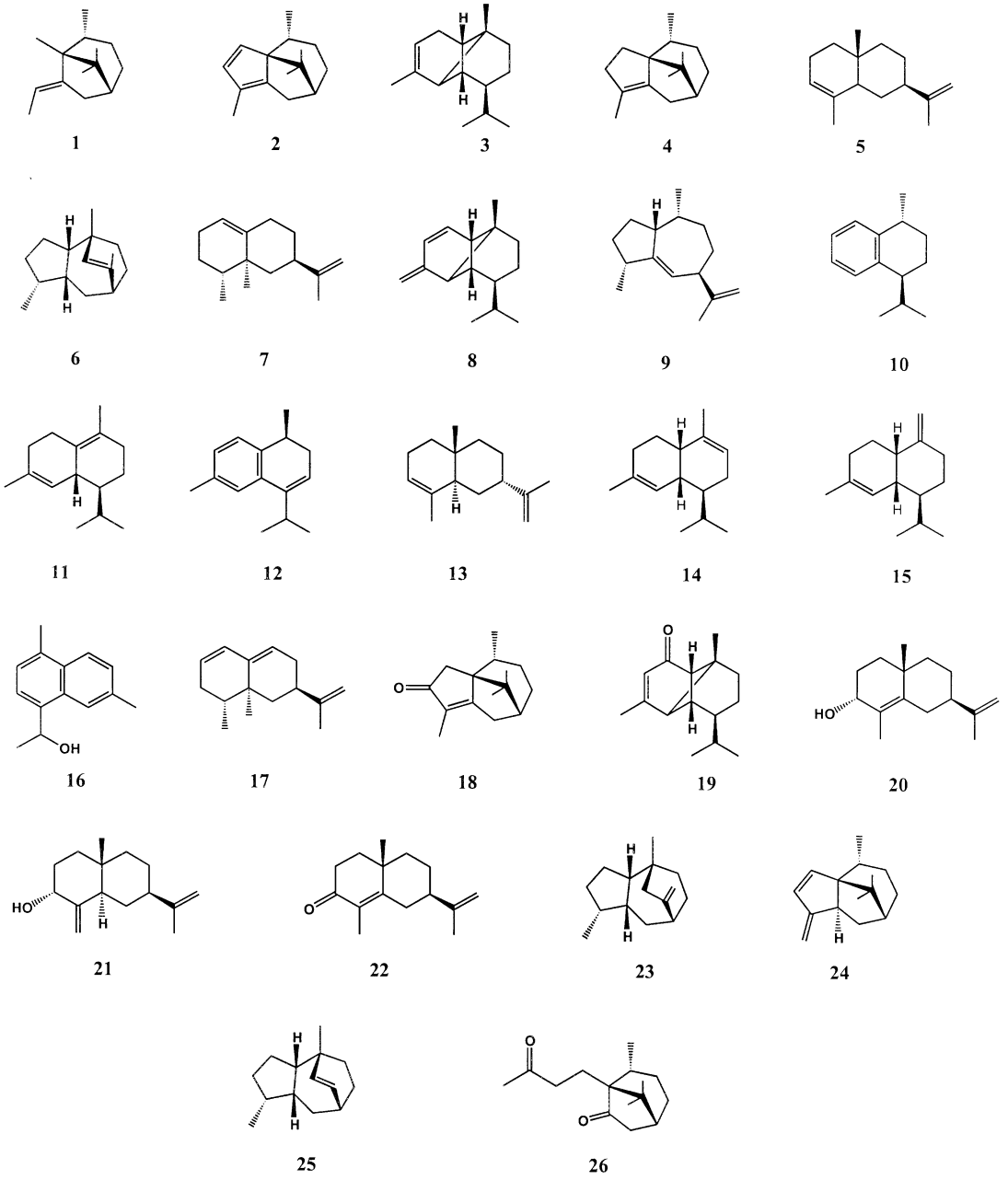


Fig. 1. Sesquiterpenes from the essential oil of *Cyperus rotundus*.

was concluded. The $^{1}H$ NMR and $^{13}C$ NMR signal assignments, achieved by $^{1}H$–$^{1}H$-COSY and HMQC, correlations, afforded three substructures. The methine proton CH-5 ($\delta$ 1.99) couples with the methine proton CH-4 ($\delta$ 1.90) which is vicinal to methyl group $CH_3$-15 ($\delta$ 0.86). The latter methine proton also couples with the methylene group $CH_2$-3 ($\delta$ 1.22 and 1.68) itself coupled to another methylene group $CH_2$-2 ($\delta$ 1.46 and 1.52) which further couples with the methine proton CH-1 ($\delta$ 1.81) giving the partial structure–CH–CH($CH_3$)–$CH_2$–$CH_2$–CH–. The methine proton $\delta$ 1.99 also couples with a methylene group $CH_2$-6 ($\delta$ 1.00 and 1.70) itself coupled to the methine proton CH-7 ($\delta$ 2.52). The proton H-7 also couples with the methylene group $CH_2$-8 ($\delta$ 1.05 and 1.71) which again couples with $CH_2$-9 ($\delta$ 1.44 and 1.72) to give the second substructure–CH–$CH_2$–CH–$CH_2$–$CH_2$–. The methylene group $CH_2$-11 ($\delta$ 2.14 and $\delta$ 2.27) and the olefinic proton at $\delta$ 5.85 couple with each other and give the third substructure–$CH_2$–C=$CH_2$. The proton-carbon long-range coupling correlations derived from the HMBC spectrum allowed to identify the skeleton of the compound. Long-range coupling correlations were found between the protons H-11, H-13 and carbon C-7 as well as between the methine proton H-7 and the carbons C-11, C-12 and C-13, indicating a bond connecting C-7 and C-12. The

proton H-11 also correlated with the carbons C-1, C-9, C-10 and C-14 while the methyl group H-14 correlates with the carbons C-1, C-9, C-10 and C-11. From these observations it was deduced that C-10 is connected to C-11, C-14 to C-10, and C-10 to C-1. Long-range correlations were observed between the protons H-6, H-5, H-4 and carbon C-1 as well as between H-1 and the carbons C-2, C-4, C-5, C-6 and C-10 indicating a bond between C-1 and C-5. The combination of all these informations led to a rotundane skeleton with an exocyclic double bond. The relative configuration at the chiral centres C-1, C-4, C-5, C-7 and C-10 was concluded from the NOESY diagram. The correlations observed between protons H-1, H-4 and H-5 indicate that these protons are on the same side of the molecule. Furthermore, the protons H-1 and H-5 show spatial interactions with the proton H-11a, which is only possible if H-1 and H-5 are located at the same side as the C-11-C-12 bridge. Therefore, compound **23**, which was named isorotundene, has the relative configuration as shown in Fig. 2.

To verify the rotundane skeleton a partial synthesis of **23** by oxymercuration-demercuration (Brown and Geoghegam, 1970) of rotundene (**6**) to isorotundenol (**27**) and subsequent dehydration (Greenwood et al., 1968) to isorotundene (**23**) and rotundene (**6**) was performed (Fig. 3). The synthesized and the isolated compounds **23** have identical spectroscopic data. Although rotundene (**6**) has been known for a long time its stereochemistry was not known as yet. The relative configuration of rotundene could not be derived from its NOESY diagram because of the overlapping of all the important signals. However, the fact that isorotundene (**23**) could be obtained from rotundene as shown in Fig. 3, proves that rotundene and isorotundene have the same relative configuration.

The identity of the relative configuration of rotundene (**6**) and isorotundene (**23**) still leaves the question about the absolute configuration open. In order to determine the absolute configuration of the two compounds, a chemical transformation of rotundene was performed in order to correlate it with (+)-γ-gurjunene (**9**). Rotundene (**6**) was submitted to ozonolysis followed by reduction with dimethyl sulfide under nitrogen (McMurry and Bosch, 1987). The desired aldehyde was not stable and was oxydised directly the to ketoacid **28** which could be isolated. The acid **28** was then decarboxylated (Fristad et al., 1983) to give ketone **29**. Subsequently **29** was allowed to react with methylenetriphenylphosphine in dimethyl sulfoxide (Greenwald et al., 1963) to yield the hydrocarbon **30** which was hydrogenated (Harmon et al., 1969) to give compound **31** (Fig. 4). Compound **31** was finally compared by enantioselective gas chromatography with the fully hydrogenated products of (+)-γ-gurjunene (**5**) and proved to have the same retention time with the major hydrogenation product (Fig. 5). The co-injection was made in a column which separates (+)- and (−)-γ-gurjunene (Fig. 6). Unfortunately, the small quantity of (−)-γ-gurjunene (which we isolated from the liverwort *Marchantia polymorpha*) was insufficient for hydrogenation and comparison with **31**. However, it is very probable that the used column also would have separated the hydrogenation products of the two enantiomers. Therefore, it is very likely that rotundene has the absolute configuration as shown for **23**.

### 2.2. (−)-Cypera-2,4(15)-diene (24)

The isolation of cypera-2,4(15)-diene (**24**) was achieved by a combination of column chromatography and preparative GC. The compound was assigned the molecular formula $C_{15}H_{22}$ ($[M]^+$ at $m/z = 202$). The $^{13}C$ NMR spectrum combined with the DEPT technique revealed three methyl groups, four methylene groups, one being olefinic (δ 102.84), five methine groups, two of which are olefinic (δ 133.26 and 139.17) and three quaternary carbon atoms, one of which is olefinic (δ 148.94).

The proton NMRspectrum displayed some signals common to patchoulane sesquiterpenes, namely the two

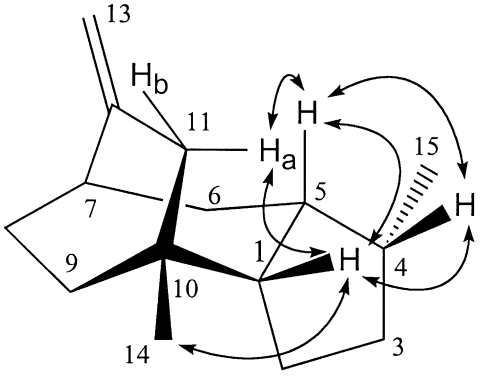


Fig. 2. Relative configuration of isorotundene with relevant NOEs.

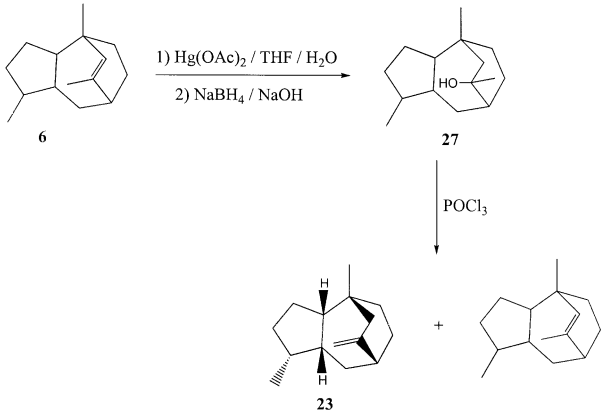


Fig. 3. Preparation of isorotundene (**23**) from rotundene (**6**).

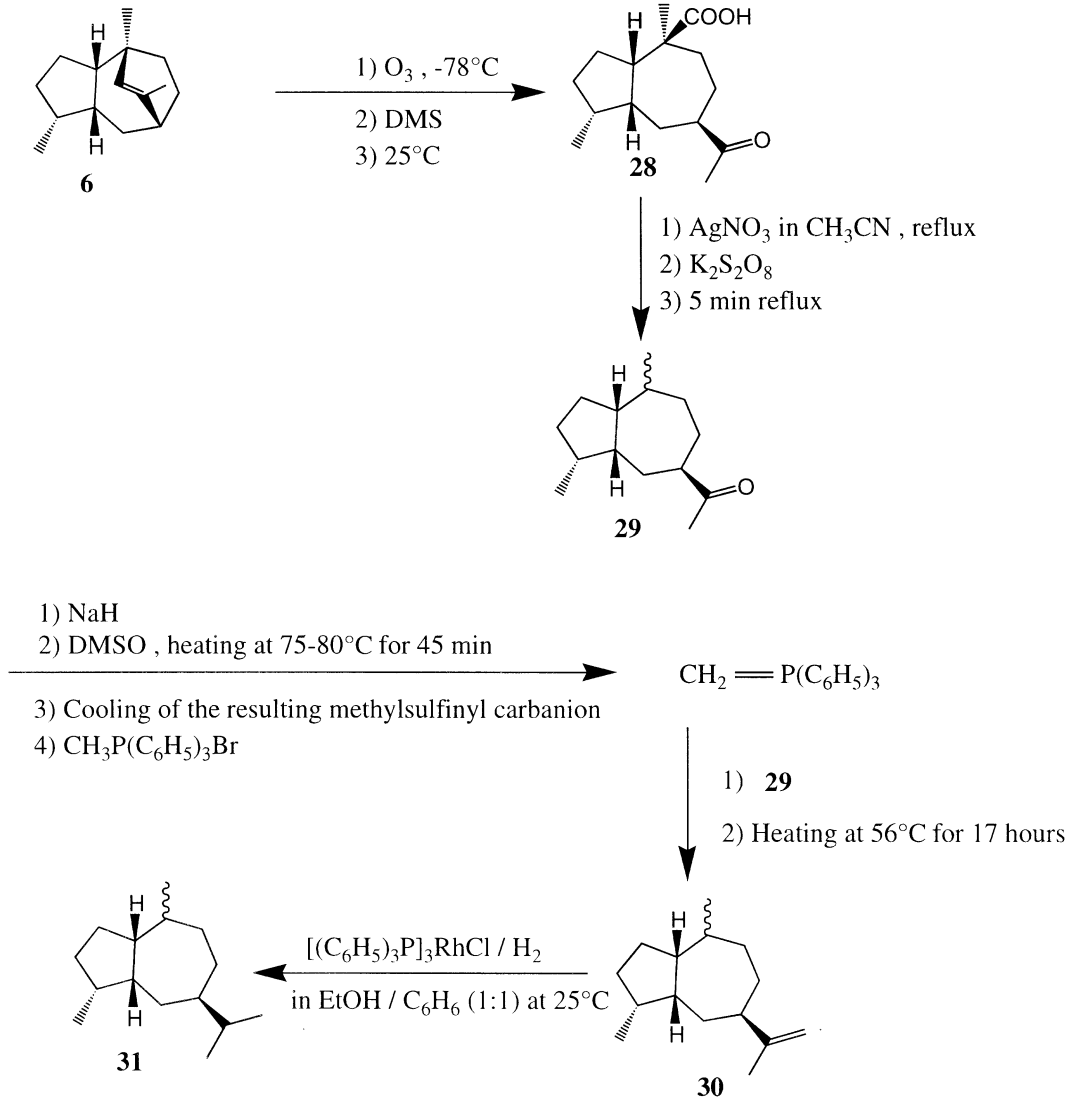


Fig. 4. Chemical conversion of the rotundane to a guaiane skeleton.

geminal methyl groups H-12 and H-13 (δ 0.99 and 1.01) which are coupled to each other (W-coupling), the methyl group at $\delta$ 0.77, appearing as a doublet and coupling with the methine proton H-10 ($\delta$ 2.12). The vinylic proton H-2 (δ 6.17) couples with another vinylic proton H-3 ($\delta$ 5.69), itself coupled to the olefinic methylene group H-15 ($\delta$ 4.98) and also to the methine proton H-5 ($\delta$ 3.03) giving the substructure –CH=CH–C(CH)=$CH_2$. Moreover, the coupling constant between H-2 and H-3 ($J = 5.60$ Hz) indicates that they belong to a five-membered ring. The methine proton H-5 also couples with the methylene group $CH_2$-6 ($\delta$ 1.68 and 2.10) which further couples with the methine proton H-7 (δ 1.85) itself coupled to the methylene group $CH_2$-8 (δ 1.51 and 1.86) which again couples with the methylene group $CH_2$-9 (δ 1.03 and 1.29) itself coupled to the methine proton CH-10 (δ 2.12). From these informations the substructure CH–$CH_2$–CH–$CH_2$–$CH_2$–CH– is derived.

All these informations from the $^{13}C$ NMR and DEPT as well as the $^1H$–$^1H$-COSY led us to propose structure **24** for the compound. Its relative configuration resulted from the NOESY diagram which shows NOE correlations between H-5, H-9 and H-14 at one side and between H-13 and H-10 at the other. The observed correlations indicate that the methine proton H-10 is on the same side with the bridge C-1–C-7, while H-5, H-9 and H-14 are located at the opposite side of the molecule (Fig. 7). **24** was before found as a minor product during the reduction of cyperotundone (**18**) to the corresponding alcohol and subsequent dehydration to **2** (Fig. 8) (Mekem Sonwa et al., 1997).

### 2.3. (−)-Norrotundene (25)

The isolation of **25** was performed by the combination of many separation methods including CC at low temperature (−20° C), CC over silver nitrate precoated

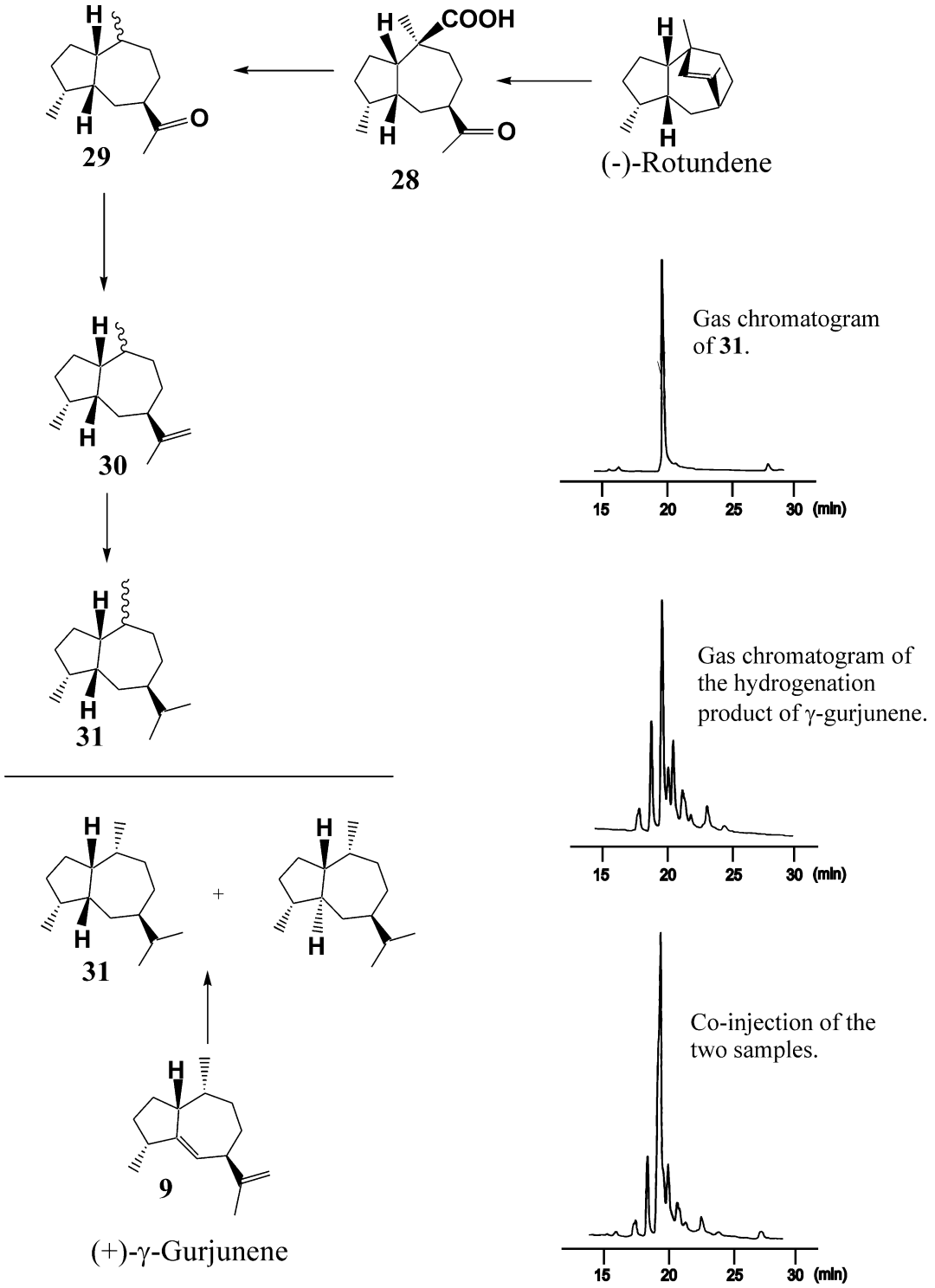


Fig. 5. Chemical transformation of rotundene and GC comparison with the hydrogenation product of (+)-γ-gurjunene.

silica, preparative TLC and GC. The compound has the molecular formula $C_{14}H_{22}$ in agreement with its mass spectrum ($[M]^+$ at $m/z$ =190). The $^{13}C$ NMR and DEPT spectra revealed 14 carbon atoms, two methyl groups, five methylene groups, six methine groups from which two are olefinic ($\delta$ 129.15 and 139.10) and one quaternary carbon atom. Therefore the compound should consist of three rings since it has a total of four unsaturations, one being a double bond. The constitution of **25** was derived from interpretation of the $^1H$ NMR spectrum and the phase-sensitive $^1H$–$^1H$-COSY spectrum together with the HMQC and the HMBC diagrams.The methine proton CH-1 ($\delta$ 2.20) couples with the methylene group $CH_2$-2 ($\delta$ 1.61 and 1.54) which

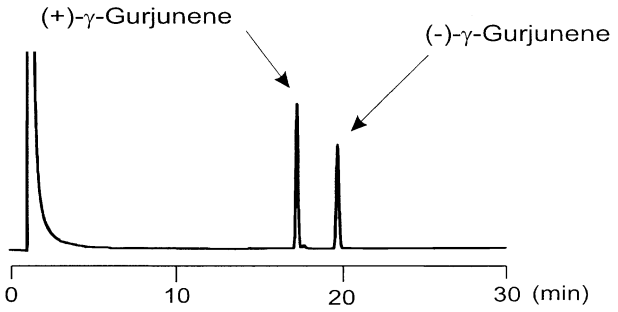


Fig. 6. Separation of (−)- and (+)-γ-gurjunene by capillary gas chromatography on a 25 m capillary column with heptakis(6-*O*-*t*.butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (50% in OV 1701, w/w) at 100 °C.

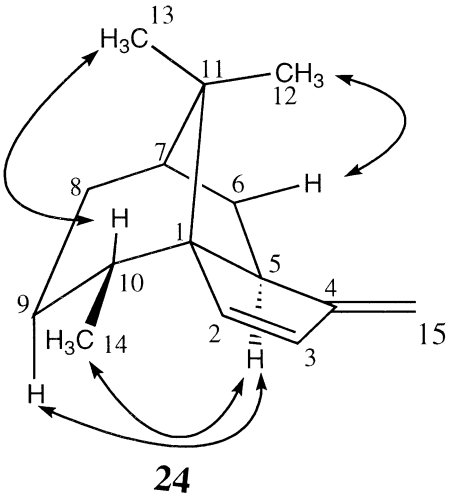


Fig. 7. Stereostructure of cypera-2,4(15)-diene (**24**) and important NOEs.

further couples with another methylene group $CH_2$-3 (δ 1.42 and 1.87) which couples again with the methine proton H-4 (δ 2.10) itself coupled to the methyl group $CH_3$-14 (δ 0.90) and the methine proton CH-5 (δ 1.80) which further couples with the methine proton H-1 (δ 2.20) and gives rise to a five-ring partial structure *a*: $C_1H$–$CH_2$–$CH_2$–$CH(CH_3)$–CH–$C_1H$–.

The methine proton H-5 also couples with methylene group $CH_2$-6 (δ 0.95 and 1.66) which further couples with the methine proton H-7 (δ 2.56), itself coupled to the methylene group H-8 (δ 1.12 and 1.91) which couples again with the methylene group H-9 (δ 1.52 and 1.83) to give the substructure *b*: CH–$CH_2$–CH–$CH_2$–$CH_2$–. Moreover, the vinylic protons H-11 and H-12 (δ 6.02) are coupled to the methine proton H-7, indicating a partial structure *c*: –CH=CH–CH–. Observing that the substructures *a* and *b* have C-5 in common and that *b* and *c* have C-7 in common, the three partial structures can be connected to give a new substructure A (Fig. 9). It may be assumed that the only tertiary methyl group of the molecule $CH_3$-13 is linked to the only quaternary carbon and thus yielding substructure B: $CH_3$–C. This assumption is confirmed by the HMBC diagram which shows long-range correlations between the protons H-13 and C-1, C-10 and C-11. Proton H-1 shows long-range connectivities to C-2, C-5, C-10 and C-11. The protons of methylene group $CH_2$-9 show connectivities to C-1, C-8, C-10 and C-11, while the olefinic proton H-11 is long-range coupled to C-1, C-7, C-9, C-10, C-12 and C-13. All these observations imply the two carbon-carbon connections between C-10–C-1 and C-10–C-11, leading to a rotundane skeleton for **25** (Fig. 9).

The relative stereochemistry at the chiral centres of the molecule could not be derived from the NOESY spectrum. The signals of the two methine protons H-1 and H-4 overlap and hence it is not possible to know which one of them is responsible for the NOE with the proton H-5. However, using the phase-sensitive COSY technique as well as the gradient selected HMQC method, it was possible to calculate the scalar coupling constant between H-4 and H-5 ($J = 5.85$ Hz). This value of the coupling constant corresponds (Karplus curve) to an angle of $\Phi = 35°$ between the two protons, which means that they are on the same side of the molecule. Accordingly, both of them have spatial interactions with H-1. It remains to investigate the stereochemistry at C-7 and C-10. To solve this problem the NOESY diagram was of no help because the protons H-1 and H-5 are too far from the vinylic protons H-11 and H-12. The solution came from the $^1$H–$^1$H-COSY diagram where a long-range coupling correlation is found between proton H-1 and one of the H-9 protons. This is a case of $^4J$ coupling through a σ-bond which requires a particular geometry (W arrangement of the two protons) for the molecule, and this is only possible if the H-1 proton and the C-11–C12 bridge are on the same side of the molecule. Moreover, the axial–axial coupling between H-5 and H-6a is only possible for this stereochemistry of the molecule (Fig. 10). Norrotundene is probably biogenetically related to rotundene and should have the same absolute configuration.

### 2.4. Cyperadione (*26*)

Cyperadione (**26**) was isolated from the oxygenated fraction of *C. rotundus* oil by preparative GC. The compound has the molecular formula $C_{15}H_{24}O_2$ ($[M]^+$ at $m/z = 236$). The $^{13}$C NMR- and the DEPTspectrum indicate that it has four methyl groups, two methine protons and four quaternary carbon atoms, two of which being carbonyl carbons (δ 208.50 and 220.25). From the $^1$H NMR and the $^1$H $^1$H-COSY spectra, three substructures were assigned which helped to derive the full structure of the product. The methyl group $CH_3$-14 (δ 0.78) couples with the methine proton CH-10 (δ 2.10) which also couples with the methylene group $CH_2$-9 (δ 1.10 and 1.68) itself coupled to the methylene group $CH_2$-8 (δ 1.45 and 2.01) which further couples with the

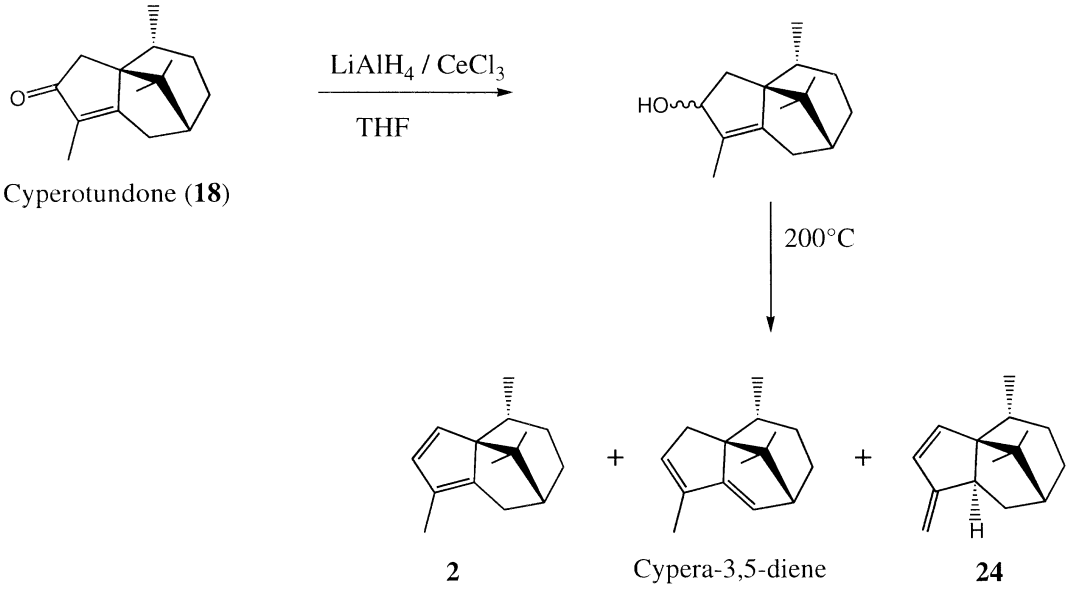


Fig. 8. Formation of cypera-2,4(15)-diene (**24**) form cyperotundone (**18**).

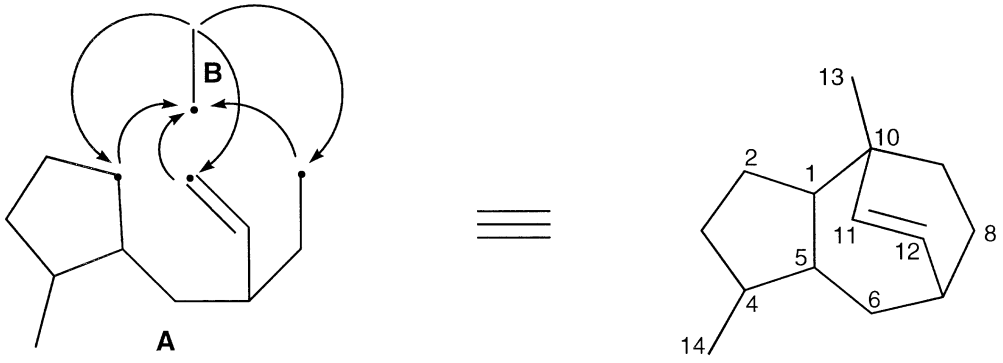


Fig. 9. Important proton-carbon long-range correlations in the HMBC spectrum of norrotundene (**25**).

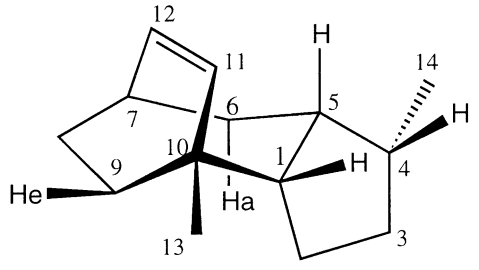


Fig. 10. Relative configuration of norrotundene (**25**). Note the W-arrangement between H-1 and H-9e, and the axial-axial disposition between H-5 and H-6a. These two geometrical patterns which are supported by the observed coupling correlations are only possible for the given stereostructure of the molecule.

methine proton CH-7 ($\delta$ 1.90) itself coupled to the methylene group $CH_2$-6 ($\delta$ 1.96 and 2.48). It should be pointed out that the signals of this methylene group appearing at lower field and showing no other coupling correlation to a proton may be adjacent to a carbonyl group. This is confirmed by the HMBC diagram where correlations are observed between the protons H-6 and carbonyl carbon C-7. From all this information substructure $CH_3$–CH–$CH_2$–$CH_2$–CH–$CH_2$–CO can be derived. The two methyl groups at $\delta$ 0.98 and 1.28 appearing as singlets are coupled to each other (W coupling), what indicates that they are linked to the same quaternary carbon atom giving the substructure $CH_3$–C–$CH_3$. The deshielded methyl group $CH_3$-15 at $\delta$ 2.18 is probably adjacent to a carbonyl function (this is also confirmed by the HMBC spectrum) and shows a long-range coupling correlation to the methylene group $CH_2$-3 ($\delta$ 2.35 and 2.44) which further couples with the methylene group $CH_2$-2 ($\delta$ 1.50 and 2.11) to give the partial structure $CH_3$–CO–$CH_2$–$CH_2$–. Additional structural informations were drawn from the HMBC diagram. The methylene protons H-2 are long-range correlated to carbons C-1, C-3, C-4, C-5, C-10 and C-11 while the methine proton H-10 shows connectivities to C-1, C-2, C-5, C-9, C-11 and C-14. From these observations the

three carbon–carbon connections C-1–C-2, C-1–C-10 and C-1–C-5 were deduced. Moreover, the methyl protons H-12 and H-13 are long-range correlated to C-1, C-7 and C-11, establishing the two connections C-11–C-1 and C-11–C-7. All these considerations led to structure **26** (Fig. 11), with a skeleton related to the patchoulane skeleton of cyperene (**4**). To confirm structure **26** we performed an ozonolysis of cyperene (**4**) (Fig. 12). The reaction product displayed the same spectral data as the isolated compound.

### 2.5. Biogenesis of patchoulane and rotundane type sesquiterpenes

Patchoulane and rotundane sesquiterpenes are characteristic for the genus *Cyperus*. Cyperene (**4**) is always the major hydrocarbon component in *Cyperus* species. Rotundene (**6**) also is always present as a major sesquiterpene hydrocarbon component. These two compounds appear to be of some chemotaxonomic importance. We have previously reported the isolation of cyprotene (**1**), a norsesquiterpene biogenetically related to cyperene from the essential oil of *Cyperus alopecuroides*. Here, we described a new norsesquiterpene, norrotundene (**25**), which most likely has a biogenetic relationship to rotundene. Besides **1** compound **25** was also identified in *C. papyrus*. It, therefore, seems to be a peculiarity of *Cyperus* species to produce norsesquiterpenes.

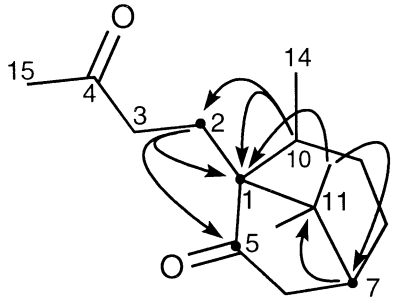


Fig. 11. Long-range correlations from the HMBC spectrum of cyperadione (**26**).

The biosynthesis of cyperene and rotundene may involve two successive cyclisation steps of farnesyl diphosphate FDP leading via a cyclodecadienyl to a cationic azulane type intermediate. At this stage two pathways *a* and *b* may take place: Path *a* proceeds by a 1,2- proton shift followed by a deprotonation leading to α-guaiene from which cyperene is formed by subsequent protonation, cyclisation and deprotonation. Path *b* proceeds by ring formation followed by a deprotonation yielding rotundene or isorotundene (Fig. 13).

## 3. Experimental

### 3.1. GC-MS

Electron impact (70 eV) GC–MS measurements were carried out on a Hewlett-Packard HP 5890 gas chromatograph equipped with a 25 m polydimethylsiloxane (Chrompack CP Sil 5 CB) capillary column and coupled to a VG Analytical VG 70-250S mass spectrometer. Helium was used as carrier gas.

### 3.2. NMR spectroscopy

NMR spectra were recorded using a Bruker DRX 500 ($^1$H: 500 MHz, $^{13}$C: 125 MHz) and a Bruker DRX 400 ($^1$H: 400 MHz, $^{13}$C: 100 MHz). Tetramethylsilane (TMS) was used as reference signal ($\delta = 0.00$).

### 3.3. Capillary GC

Orion Micromat 412 double column instrument with 25 m fused silica capillaries with CPSil 5 and CPSil 19 (Chrompack); Carlo Erba Fractovap 2150, 4160 instruments with 25 m fused silica capillaries with heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β- (2,6-Me-3-Pe-β-CD) and - γ - cyclodextrin, respectively, and heptakis(6 - *O* - t.butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin (6T-2,3-Me-β-CD), all in polysiloxane OV1701 (50 wt%); split injection, flame ionization detection; carrier gas 0.5 bar hydrogen.

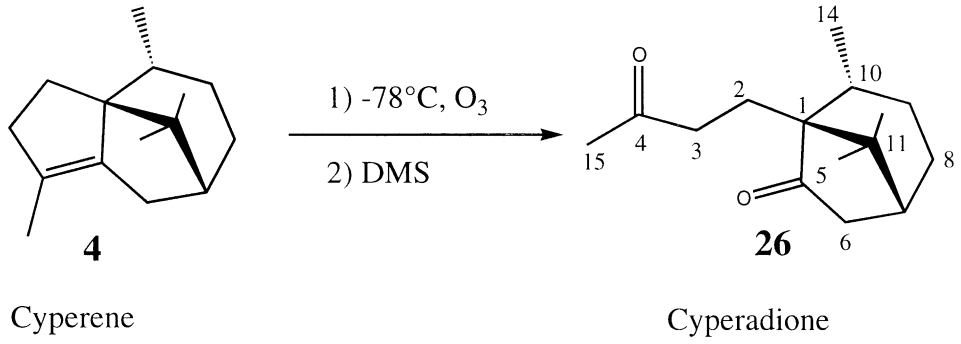


Fig. 12. Preparation of cyperadione by ozonolysis of cyperene.

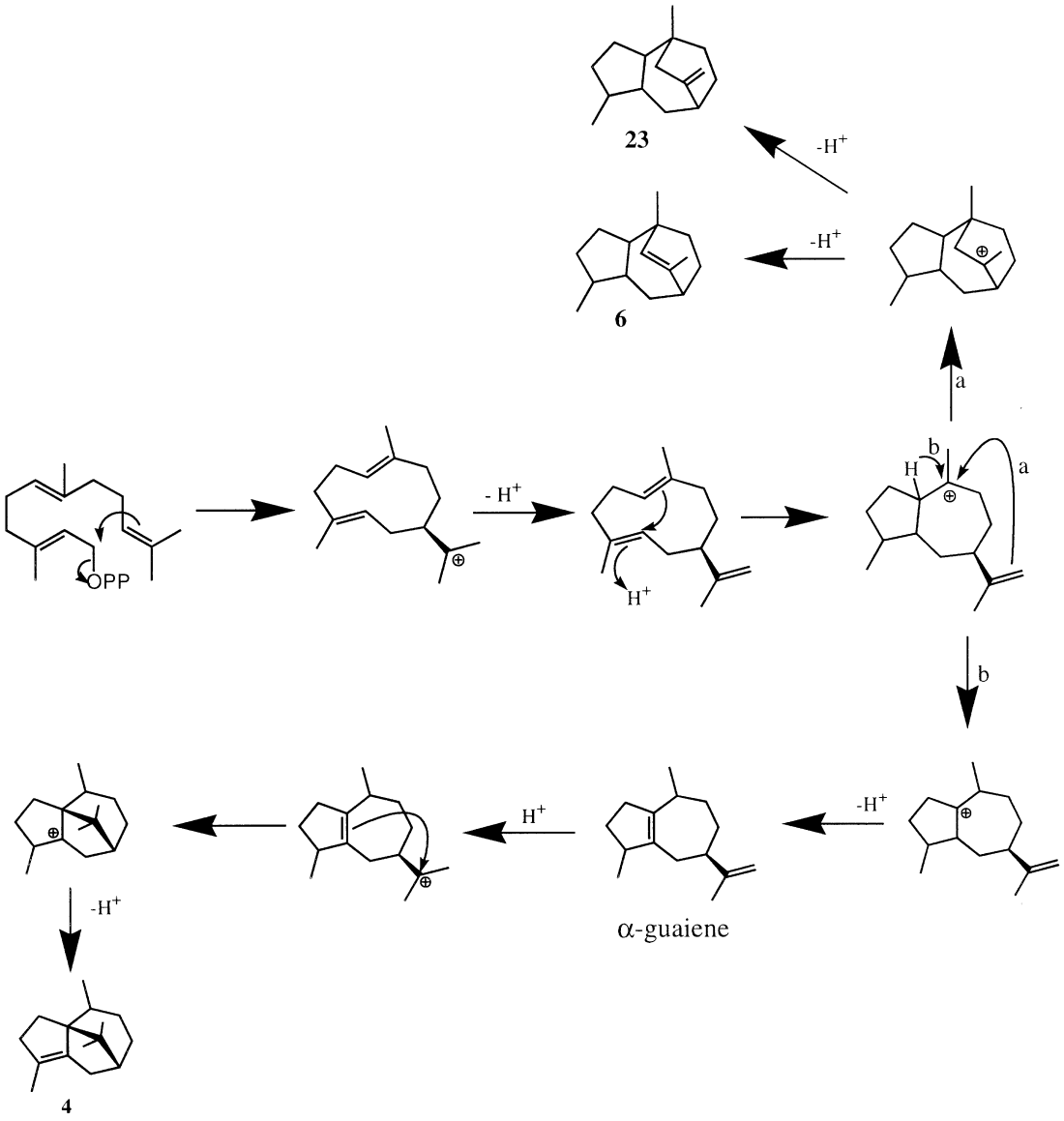


Fig. 13. Proposed biogenetic pathway for cyperene, rotundene and isorotundene.

### 3.4. Column chromatography at low temperature

Prefractionations of essential oils were performed on a silica gel column equipped with a condenser and connected to a Kryoflex KF 40 cooling system from Messgeräte-Werk Lauda at −20 °C. Ethanol was used as cooling liquid.

### 3.5. Thin layer chromatography

Thin layer chromatography was effected using glass and aluminum plates of silica 60 $F_{254}$ (Merck). Silver nitrate coated plates were prepared by immersing the plates into an ethanol–water (4:1) solution of silver nitrate (5 mg $AgNO_3$ in 50 ml of solvent). After 30 min the plates were removed from the solution and dried in an oven. An ethanolic solution of sulfuric acid (10%) was used as a spray reagent.

### 3.6. Essential oil

The essential oil of *C. rotundus* was a gift of K.-D. Protzen, Paul Kaders GmbH, Hamburg.

### 3.7. Isolation of compound **23**

A preliminary separation of the essential oil was performed by column chromatography at low temperature (−20 °C). This resulted in a fractionation of the oil into a hydrocarbon and an oygenated fraction. One gram of the hydrocarbon fraction was then separated by TLC using petroleum ether as eluting solvent. The aim of this

separation was to eliminate the major hydrocarbon (cyperene) from the sample before any other operation. Three bands were obtained, the first two containing mainly cyperene and α-copaene. Only the third fraction ($R_f = 0.45$) was interesting because it contained the unknown products. This fraction was further separated by preparative GC (column 6T-2,3-Me-β-CD, temperature programme: from 110 to 180 °C, heating rate 2°/min). Fraction 5 of the chromatogram contained (+)-ylanga-2,4(15)-diene (**8**). Fraction 8 was analysed by GC–MS and three major products were present: α-selinene (**5**), valencene (**7**), and an unknown product. This sample was further separated by preparative TLC on silver nitrate precoated plates, using petroleum ether as eluting solvent. Two bands were obtained, the second of which ($R_f = 0.35$) was taken up in petroleum ether and purified by preparative GC. Spectroscopic analysis resulted in the structure of isorotundene **23**.

### 3.8. (−)- Isorotundene (*23*)

$^1$H NMR (500 MHz, $C_6D_6$): see Table 1. $^{13}$C NMR (100 MHz, $C_6D_6$): δ 16.10 (*q*, C-15), 25.20 (*t*, C-2), 27.53 (*t*, C-8), 29.28 (*t*, C-9), 31.48 (*t*, C-3), 32.28 (*q*, C-14), 33.15 (*t*, C-6), 34.38 (*s*, C-10), 38.25 (*d*, C-4), 38.64 (*d*, C-7), 41.52 (*d*, C-5), 44.36 (*t*, C-11), 56.19 (*t*, C-1), 107.98 (*t*, C-13), 151.08 (*s*, C-12). MS (EI, 70 eV), *m*/*z* (rel. int.) : 204 (33) $[M]^+$, 189 (84), 175 (25), 161 (64), 144 (45), 133 (41), 119 (54 ), 108 (100), 93 (91), 79 (71), 67 (46), 55 (51), 41 (82).

Table 1
$^1$H NMR spectral data for compounds **23** and **25**[a]

| H | 23 | 25 |
|---|---|---|
| 1 | 1.81 *ddd* (9.5, 9.5, 5.9) | 2.20 *dddd* (13.5, 6.6, 6.4, 7.0) |
| 2 | 1.46 *dd* (9.5, 3.2) | 1.54 *m* |
| | 1.52 *m* | 1.61 *m* |
| 3 | 1.22 *dddd* (13.6, 11.4, 11.4, 6.7) | 1.42 *dddd* (13.5, 11.0, 11.0, 6.7) |
| | 1.68 *m* | 1.87 *m* |
| 4 | 1.90 *dddd* (13.6, 6.9, 6.6, 6.4) | 2.10 *m* |
| 5 | 1.99 *dddd* (13.2, 6.4, 6.4, 5.9) | 1.80 *dddd* (13.2, 6.4, 6.4, 6.4) |
| 6 | 1.00 *t* (13.2) | 0.95 *dd* (13.2, 13.2) |
| | 1.70 *m* | 1.66 *ddd* (13.2, 9.1, 6.4) |
| 7 | 2.52 *m* | 2.56 *m* |
| 8 | 1.05 *dd* (12.9, 9.5) | 1.12 *ddd* (13.8, 11.7, 5.1) |
| | 1.71 *m* | 1.91 *ddd* (13.8, 9.4, 5.0) |
| 9 | 1.44 *m* | 1.52 *ddd* (11.7, 9.4, 6.1) |
| | 1.72 *m* | 1.83 *m* |
| 11 | 2.14 *dt* (17.0, 2.5, 2.5) | 6.02 *m* |
| | 2.27 *dd* (17.0, 2.2) | |
| 12 | | 6.02 *m* |
| 13 | 5.85 *m* | 1.13 *s* |
| | 5.85 *m* | |
| 14 | 0.80 *s* | 0.99 *d* (6.6) |
| 15 | 0.86 *d* (6.6) | |

[a] All the measurements were done in $C_6D_6$. The assignments were established by $^{13}$C–$^1$H HMBC and HMQC

### 3.9. Preparation of isorotudene (*23*) from rotundene (*6*)

#### 3.9.1. Hydration of rotundene by oxymercuration-demercuration

In a 50 ml flask, fitted with a magnetic stirrer, 48 mg of mercuric acetate were placed. To this, 3 ml of water were added followed by 3 ml of THF. After addition of 30 mg (0.148 mmol) of rotundene the reaction mixture was stirred for 15 min at room temp. to complete the oxymercuration. 3 ml of 3.0 M sodium hydroxyde were added followed by 3 ml of a solution of 0.50 M sodium borohydride in 3.0 M sodium hydroxide. Reduction of the mercurial complex is almost instantaneous. The mercury is allowed to precipitate. Sodium chloride was added to saturate the water layer. The upper layer of THF was removed and purified by preparative GC, yielding 26 mg (80%) of isorotundenol (**27**).

#### 3.9.2. Isorotundenol $C_{15}H_{25}OH$ (*27*)

$^1$H NMR (500 MHz, $C_6D_6$): δ 0.75 (3H, *d*, $J = 6.61$ Hz), 0.76 (3H, *s*), 1.02 (3H, *s*), 1.08-1.54 (12H, *m*), 1.57–1.65 (2H, *m*), 1.75 (1H, *m*), 2.15 (1H, *m*). MS (EI, 70 eV), *m*/*z* (rel. int.) : 222 (5), 204 (19), 189 (27), 175 (7), 161 (14), 147 (12), 133 (13), 121 (33), 108 (100), 93 (57), 81 (39), 67 (24), 55 27), 41 (35).

#### 3.9.3. Dehydration of isorotundenol

Phosphoryl chloride (0.5 ml) was added to a pyridine solution (1 ml) of isorotundenol (10 mg). The mixture was stirred for 10 h and pyridine was then removed by distillation at low pressure. The remaining residue was taken up in hexane and separation by prep. GC yielded 3 mg of isorotundene and 3 mg of rotundene.

### 3.10. Ozonolysis of rotundene

A solution of rotundene (50 mg) in dichloromethane (20 ml) was treated at −78° C with a stream of ozone until a persistant blue colour was observed. The solution was then purged with nitrogen for 5 min. After addition of dimethylsulfide (3 ml) the solution was washed with ice-cold sodium bicarbonate solution (25 ml), water and brine. The organic layer was dried ($NaSO_4$) and the solvent was evaporated. Purification of the residue by prep. GC gave the keto-acid **28** (45 mg).

$^1$H NMR (400 MHz, $CDCl_3$): δ 1.10 (3H, *d*, $J = 6.61$ Hz), 1.30 (1H, *m*), 1.35 (3H, *s*), 1.65–1.92 (9H, *m*), 2.11 (1H, *m*), 2.20 (1H, *m*), 2.29 (3H, *s*), 2.58–2.85 (2H,*m*). $^{13}$C NMR (100 MHz, $CDCl_3$): 16.73, 25.10, 25.66, 27.47, 28.30, 30.07, 31.09, 31.60, 39.64, 41.70, 48.56, 48.64, 50.55, 184.59, 212.01. MS (EI, 70 eV) *m*/*z* (rel. int): 252 (13) $[M]^+$, 234 (7), 206 (15), 191 (11), 163 (36), 149 (7), 133 (10), 121 (18), 107 (25), 95 (68), 81 (43), 67 (22), 55 (28), 43 (100).

### 3.11. Decarboxylation of keto-acid **28**

The keto-acid (40 mg, 0.16 mol) and silver nitrate (1 mg) were dissolved in acetonitrile (6 ml) and water (2 ml) and heated to reflux. To this solution potassium peroxosulfate ($K_2S_2O_8$) (0.32 mg) in water (5 ml) was slowly added. Refluxing was continued for another 5 min before the reaction mixture was cooled and extracted with a saturated sodium bicarbonate solution (10 ml, three times), dried ($MgSO_4$) and purified by prep. GC to give 14 mg of ketone **28**.

$^1$H NMR (500 MHz, $CDCl_3$): 0.96 (3H, *dd*, $J=6.62$ Hz), 1.06 (3H, *s*), 1.40 (1H, *m*), 1.60–1.84 (7 H, *m*), 1.97–2.1 (2H, *m*), 2.13–2.24 (3H, *m*), 2.27 (3H,*s*), 2.44 (1 H, *m*), 2.69 (1H, *dd*, $J_1=13.87$ Hz, $J_2=6.94$ Hz). $^{13}$C NMR (100 MHz, $CDCl_3$): 15.10 (*q*), 21.52 (*q*), 26.09 (*t*), 26.80 (*t*), 27.85 (*q*), 30.26 (*t*), 33.25 (*t*), 37.46 (*t*), 37.99 (*d*), 38.04 (*d*), 46.82 (*d*), 56.70 (*d*), 61.65 (*d*), 207.93 (*s*). MS, (EI, 70 eV), *m*/*z* (rel. int): 208 (5) $[M]^+$, 190 (7), 175 (6), 161 (5), 150(8), 135 (9), 123 (27), 109 (23), 95 (100), 81 (31), 67 (25), 55 (29), 43 (52).

### 3.12. Reduction of ketone **29**

In a three-necked flask sodium hydride (0.45 mmol) was washed with several portions of pentane to remove the mineral oil. The flask was then equipped with rubber stopper, a reflux condenser and a magnetic stirrer. The system was alternately evacuated and filled with nitrogen; 3 ml of dimethyl sulfoxide was introduced via a syringe and the mixture was heated at 75–80° C for 45 min. The resulting solution of methylsulfinyl carbanion was cooled in an ice-water bath, and 108 mg of methyltriphenylphosphonium bromide in 100 ml of warm dimethyl sulfoxide were added. The resulting dark red solution of the ylide was stirred at room temperature for 10 min before 7 mg of the ketone **29** in two ml of dimethyl sulfoxide were added. The reaction mixture was then heated at 56°C for 16 h after which the solution was allowed to cool and 10 ml of water were added. The two phases were extracted three times with *n*-pentane (40 ml). The *n*-pentane fractions were combined and washed with 10 ml of a (1/1) water/dimethyl sulfoxide solution and then with 30 ml of 50% saturated sodium chloride solution. The pentane layer was then dried over anhydrous sodium sulfate and purification by preparative GC gave 2 mg of the alkene **30**.

MS (EI, 70 eV), *m*/*z* (rel. int): 206 (12) $[M]^+$, 191 (18), 177 (6), 163 (51), 149 (25), 122 (39), 107 (56), 95 (60), 81 (100), 67 (57), 55 (66), 41 (79).

### 3.13. Hydrocarbon 31

a. **30** (1 mg) was dissolved in a mixture of ethanol (1 ml) and benzene (1 ml). The catalyst $[(Ph)_3P]_3RhCl$ (1 mg) was added and the solution turned orange. A gentle stream of hydrogen was then passed through the solution under normal pressure for 15 min. The solvent was removed under reduced pressure and petroleum ether was added, since most of the catalyst remains undissolved. Filtration through a column of alumina afforded the pure alkane **31**. MS (EI, 70 eV), $[M]^+$ $m/z=208$.

b. The hydrogenation of (+)-γ-gurjunene (**9**) followed the same procedure as that of the alkene **30** except the catalyst was replaced by nickel on charcoal.

### 3.14. Isolation of cypera-2,4(15)-diene and norrotundene

For the above described chemical transformation, a relatively large amount of rotundene had to be isolated from the essential oil of *C. rotundus*. For the isolation 5 g of the hydrocarbon fraction of the oil were separated by column chromatography over silver nitrate precoated silica using a petroleum ether–chloroform gradient for elution. The eluent from the column was monitored by capillary GC and 16 fractions were collected. Fractions 1–6 obtained by elution with petroleum ether contained only known hydrocarbons. Fractions 7–10 were obtained by elution with petroleum ether–chloroform (7/3) and contained the rotundene. It could be proved by GC–MS of fraction eighth that the sample also contained an unknown minor component. The separation of this fraction was performed by prep. GC (column: 2,6-Me-3Pe-β-CD; temp. programme: 100–140 °C, heating rate 2 °C/min). The chromatogram displayed five peaks the fifth containing the pure unknown product which was characterized as cypera-2,4(15)-diene (**24**). Fractions 11–15 were eluted with petroleum ether–chloroform (1/1). GC–MS revealed the presence of an unknown product of molecular weight 190 among many known sesquiterpene hydrocarbons. In order to isolate the unknown compound, the joined fractions 14 and 15 were first separated by prep. TLC over silver nitrate precoated plates, using petroleum ether–chloroform (7/3) as eluting solvent. Three bands were obtained and the third one, which contained the norsesquiterpene as major product was separated by preparative GC (column: SE 30, temp. 125 °C). Spectroscopic data permitted the characterization of the compound as norrotundene (**25**).

#### 3.14.1. (−)-Cypera-2,4(15)-diene (**24**)

$^1$H NMR (400 MHz, C6D6): see Table 2. $^{13}$C NMR (400 MHz, C6D6) : 17.32 (*q*), 19.61 (*q*), 26.36 (*q*), 26.64 (*q*), 27.70 (*t*), 28.48 (*s*), 30.16 (*t*), 32.58 (*t*), 44.96 (*d*), 47.66 (*d*), 61.93 (*s*), 102.84 (*t*), 133.26 (*d*), 139.17 (*d*), 148.94 (*s*). MS (EI, 70 eV) *m*/*z* (rel. int.): 202 (83) $[M]^+$, 187 (24), 173 (6), 173 (6), 159 (46), 145 (24), 131 (37), 118 (85), 106 (100), 91 (78), 77 (33), 69 (17), 65 (18), 55 (26), 41 (61).

#### 3.14.2. (−)-Norrotundene (**25**)

$^1$H NMR (500 MHz, C6D6):see Table 1. $^{13}$C NMR (125 MHz, C6D6) : 14.73 (*q*), 22.95 (*t*), 28.00 (*t*), 28.67

Table 2
$^1$H NMR spectral data for compounds **24** and **26**[a]

| H | **24** | **26** |
|---|---|---|
| 2 | 6.17 *d* (5.6)<br>2.11 *m* | 1.50 *ddd* (15.4, 11.7, 5.7) |
| 3 | 5.69 *dd* (5.6, 1.0)<br>2.44 *ddd* (16.4, 12.3, 5.7) | 2.35 *ddd* (16.4, 11.7, 4.4) |
| 5 | 3.03 *m* | |
| 6 | 1.68 *m*<br>2.10 *m* | 1.96 *d* (19.0)<br>2.48 *dd* (19.0, 7.3) |
| 7 | 1.85 *m* | 1.90 *ddd* (6.6, 3.3, 3.3) |
| 8 | 1.51 *dt* (14.8, 6.1)<br>1.86 *m* | 1.45 *dq* (13.6, 3.2)<br>2.01 *dddd* (13.6, 12.9, 6.0, 2.7) |
| 9 | 1.03 *m*<br>1.29 *m* | 1.10 *dddd* (14.5, 12.9, 12.9, 6.6)<br>1.68 *ddd* 14.5, 6.0, 6.0) |
| 10 | 2.12 *dddd* (13.2, 6.1, 6.1, 6.1) | |
| 12 | 0.99 *s* | 1.22 *s* |
| 13 | 1.01 *s* | 0.98 *s* |
| 14 | 0.77 *d* (6.6) | 0.78 *d* (6.6) |
| 15 | 4.98 *m*<br>4.98 *m* | 2.18 *s* |

[a] All the measurements were done in $C_6D_6$ for **24** and in $CDCI_3$ for **26**. The assignments were established by $^{13}C$–$^1H$ HMBC and HMQC

(*t*), 29.36 (*q*), 30.35 (*d*), 30.54 (*t*), 31.97 (*t*), 35.72 (*s*), 36.32 (*d*), 42.18 (*d*), 50.74 (*d*), 129.15 (*d*), 139.10 (*d*). MS (EI, 70 eV) *m*/*z* (rel. int.) : 190 (10) [M]+, 175 (15), 161 (20), 148 (7), 133 (9), 119 (7), 107 (14), 94 (100), 79 (50), 67(12), 55 (14), 41 (23).

### 3.15. Isolation of cyperadione (*26*)

The fraction of oxygenated sesquiterpenes was submitted to prep. TLC using petroleum ether–ethyl acetate (8/2) as eluting solvent. The second band ($R_f$=0.35) was then taken up in diethyl ether and submitted to further separation by prep. GC (column: 6T-2,3-Me-β-CD, 120–180 °C, 2 °C/min).

#### 3.15.1. Cyperadione (*26*)

$^1$H NMR (400 MHz, CDCl3): see Table 2. $^{13}$C NMR (100 MHz, CDCl3) : δ 16.76 (*q*), 21.87 (*t*), 22.18 (*q*), 26.42 (*t*), 27.05 (*q*), 28.10 (*t*), 30.03 (*q*), 31.32 (*d*), 38.71 (*t*), 41.79 (*d*), 41.84 (*t*), 42.96 (*s*), 58. 21 (*s*), 208.50 (*s*), 220.25 (*s*). MS (EI, 70 eV), *m*/*z* (rel. int.): 236 (66) $[M]^+$, 221 (89), 207 (7), 193 (25), 179 (63), 161 (17), 150 (20), 135 (22), 123 (22), 107 (25), 95 (19), 81 (20), 67 (23), 55 (27), 43 (100).

### 3.16. Ozonolysis of cyperene (*4*)

Three mg of cyperene in dichloromethane (20 ml) were treated at −78 °C with a stream of ozone until a persistant blue colour was observed. The solution was then purged with nitrogen for 5 mins. 1 ml of dimethylsulfide was then added, and washed with 10 ml of an ice-cold sodium bicarbonate solution, water and brine. The organic layer was dried over $MgSO_4$ and the solvent evaporated. The residue was then purified by prep. GC to give 3 mg of cyperadione (**26**).

## Acknowledgements

The financial support of DAAD (scholarship for M. Mekem Sonwa) and of the Fonds der Chemischen Industrie is gratefully acknowledged. We also thank Dr. V. Sinnwell, University of Hamburg, for his support in obtaining the NMR spectra and to A. Meiners and M. Preusse for recording the mass spectra.

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