# NEUTRAL COMPONENTS OF THE EXTRACT FROM *Homogine alpina* (L.) CASS.*

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The structures of three main sesquiterpenic components of the neutral light petroleum extract of *Homogyne alpina* (L.) CASS. have been elucidated. The first one is identical with bakkenolide A (*V*), and the remaining two are the angeloyl ester of 2-hydroxybakkenolide A (*VI*) and tigloyl ester of 3-hydroxybakkenolide A (*VII*). In addition to these substances two benzofurans have been also isolated and identified, *i.e.* euparin (*I*) and 7-methoxyeuparin (*II*).

The components of the neutral extract of the plant *Homogyne alpina* (L.) CASS. have been investigated in order to obtain further data for the corroboration of the importance of the occurrence of sesquiterpenic compounds of eremophilane type as a chemotaxonomic character in the classification of plants belonging to the *Senecioneae* tribe[1,2].

From the dry herb of the plant a neutral light petroleum extract was obtained. Chromatographic analysis showed that both the leaves and the rhizomes contained practically the same main components, and therefore only the whole plant was worked up in subsequent work. The main separation of the extract by chromatography on silica gel afforded the collective fractions $E_1$ to $E_8$ which were further separated by different procedures. Fraction $E_1$ contained a relatively large amount of hydrocarbons and waxy substances (30%). From the fractions $E_3$ and $E_5$ two substances were obtained by repeated chromatography. The first one, $C_{13}H_{12}O_3$, was yellow, with m.p. 122–123°C. An analysis of the IR spectrum, mass spectrum and $^1$H-NMR spectrum (Table I) of the natural substance and of its tetrahydro derivative indicated that it was identical with euparin[3] (*I*). The identity proof was carried out by comparison with an authentic sample[4], by spectral methods and mixed melting point determination. The second, green-yellow substance, *II*, with m.p. 95–96°C and the composition $C_{14}H_{14}O_4$ represents the methoxy derivative of euparin, which follows from its elemental analysis and from the analysis of the mass, IR and $^1$H-NMR spectra of this substance and its tetrahydro derivative (Table I).

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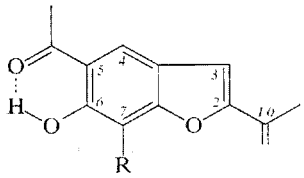
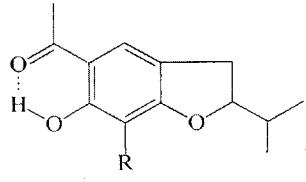
The position of the methoxy group was determined indirectly both by the determination of a nuclear Overhauser effect between the methyl protons of the acetophenone group and the closest aromatic proton of the six-membered ring, and by the hydrogenation of the furan double bond. The structure of this compound is *II*. According to the $^1$H-NMR data it is identical with the substance isolated from *Helianthella uniflora*[5].



*I*, R = H
*II*, R = $OCH_3$



*III*, R = H
*IV*, R = $OCH_3$

Fraction $E_4$ afforded a crystalline compound in high yield (20% of the extract), of m.p. 80–81°C and composition $C_{15}H_{22}O_2$. From the analysis of its IR and $^1$H-NMR spectra (Table II) it could be supposed that it is bakkenolide A (*V*) (ref.[6]). The proof was performed by comparison with an authentic sample, using IR and $^1$H-NMR spectroscopy and also mixed melting point determination. Their reduced

TABLE I
Characteristic Parameters of $^1$H-NMR Spectra of Euparin and Its Derivatives

| Substance[a] | $H_{(2)}$ | $H_{(3)}$ | $H_{(4)}$ | $H_{(7)}$ | $H_{(10)}$ |
|---|---|---|---|---|---|
| *I* | — | 6·55 bs | 7·90 s | 6·98 bs | — |
| *II* | — | 6·53 s | 7·62 s | — | — |
| *III* | 4·60 m | 2·84 dd[b,f]<br>3·15 | 7·45 t[g] | 6·30 s | 1·95[h] |
| *IV* | 4·64 m | 2·86 dd[b,f]<br>3·18 dd | 7·23 t[j] | — | 1·98[h] |

[a] Measured on a Varian HA-100 instrument, solvent deuteriochloroform, internal standard tetramethylsilane, chemical shifts in $\delta$-scale, splittings in Hz, all data from first-order analysis, multiplicity (if given) indicated as usually; [b] high-field proton indicated as $H_a$, the low-field one as $H_b$; [c] $J(CH_3, H_a) \cong 1{\cdot}5$, $J(CH_3, H_b) \neq 0 < 1$; [d] $J(CH_3, H_a) = 1{\cdot}4$, $J(CH_3, H_b) = 0{\cdot}6$,

derivatives were also identical: $C_{15}H_{24}O_2$, obtained by hydrogenation on palladium catalyst in ethyl acetate, and $C_{15}H_{26}O_2$, m.p. 81–82°C, obtained on reduction with lithium aluminum hydride.

From fraction $E_6$ we obtained two pure fractions containing substances structurally related to bakkenolide A, but in a lower yield (4·5% of the extract). From the first fraction substance *VI* was obtained, m.p. 62–64°C, which according to a detailed analysis of its IR, mass and mainly $^1$H-NMR spectra was an angeloyloxy derivative of bakkenolide A. The second fraction contained according to mass, IR and $^1$H-NMR spectral analysis substance *VII*, the structure of which corresponded to a tigloyloxy derivative of bakkenolide A. Neither of these two substances was identical with any of the known derivatives of bakkenolide A isolated so far by Japanese authors[6–11].

From the presence of the methine proton signals of RCOO—CH at 5·17 p.p.m. (*VI*) and 5·12 p.p.m. (*VII*) in the $^1$H-NMR spectra of substances *VI* and *VII* it followed that the acyloxy group is in both cases a secondary one. In the case of compounds *VI* and *VII*, however, the multiplicity of the signals of all these methine protons did not follow directly from the spectra, due to the coincidence with the signals of the exomethylene group protons of the γ-lactone ring, but it could be determined only from the $^1$H-NMR spectra of hydroxy derivatives *VIII* and *IX* obtained on alkaline hydrolysis of compounds *VI* and *VII*. The signal of the methine proton of compound *VIII* appeared as a quintet at 4·12 p.p.m. ($CDCl_3$; $J \approx 3{\cdot}1$ Hz, $\sum J \approx 13$ Hz) and in the case of compound *IX* as a triplet of doublets at 3·42 p.p.m.

TABLE I
(*Continued*)

| $C_{(10)}$—$CH_3$ | $C_{(10)}$=$CH_2$[b] | CO—$CH_3$ | $OCH_3$ | OH | Substance |
|---|---|---|---|---|---|
| 2·10 dd[c] | 5·19[c]<br>5·76 | 2·68 | — | 12·48 | *I* |
| 2·11 dd[d] | 5·21[e]<br>5·78 | 2·66 | 4·19 | 12·54 | *II* |
| 0·94 d[i]<br>1·03 d[i] | — | 2·52 | — | 12·95 | *III* |
| 0·93 d[i]<br>0·99 d[i] | — | 2·51[j] | 3·95 | 12·78 | *IV* |

$J(CH_3, H_3) \neq 0$; [e] $H_a$ q, $H_b$ bs, $^2J_{a,b} = 1{\cdot}4$, $J(H_a, H_3) \neq 0$; [f] $J(H_{3a}, H_2) = 8{\cdot}0$, $J(H_{3b}, H_2) = 9{\cdot}0$, $^2J(H_{3a}, H_{3b}) = 15{\cdot}0$; [g] $J(H_4, H_3) = 1{\cdot}5$; [h] octet, $J(H_{10}, CH_3) \cong J(H_{10}, H_2) \cong 6{\cdot}5$; [i] $J = 6{\cdot}5$; [j] $J(H_4, H_3) = 1{\cdot}2$, NOE ~ 11% for $H_4$—($CH_3CO$) experiment in nondegased solution.

($CDCl_3$; second order multiplet; $\sum J \approx 25$ Hz, $J_1 \approx J_2 \approx 10$; $J_3 \approx 4$). This multiplicity alone excluded the possibility of the presence of an OH group on $C_{(6)}$ or $C_{(9)}$, and it indicated, on the contrary, in the first approximation the presence of four vicinal interactions in the case of *VIII*, *i.e.* the fragment $CH_2$—CH(OH)—$CH_2$, and hence also the position $C_{(2)}$, and in the case of *IX* the presence of three vicinal

TABLE II

Characteristic Parameters of $^1$H-NMR Spectra of Bakkenolide A and Related Derivatives

| Compound[a] | $H_{(4)}$[b] | $H_{(6)}$[c] | $H_{(6')}$[c] | $H_{(12)}$[c] |
|---|---|---|---|---|
| *V* | 1·50 | 1·97 s | 1·97 s | 4·78 dt (12·8, 2·2) |
| *V*[d,e,f] | 1·20 | 1·92 d (13·4) | 1·70 d (13·4) | 4·15 dt (12·8, 2·2) |
| *VI*[e,g] | — | 2·01 s | 2·01 s | 4·75 t (~2) |
| *VII*[e,h,i] | — | — | — | 4·78 t (~2) |
| *VIII*[k,l] | 2·08 | 2·04 d (14·5) | 1·96 d (14·5) | 4·75 t (2·1) |
| *IX*[e] | 1·60 | 1·98 s | 1·98 s | 4·79 dt (12·8, 2·2) |
| *X*[m,n] | 2·09 | 2·02 s | 2·02 s | 4·77 t |
| *XI*[m,o] | 1·90 | 2·02 s | 2·02 s | 4·78 t |
| *XII*[p] | 2·22 | 2·10 d (14·5) | 2·01 d (14·5) | 4·76 t (2) |
| *XII*[d,q] | 1·77 | — | — | 4·07 t |
| *XIII* | 2·59 q | — | — | 4·81[r] |
| *XIII*[d] | 2·09 | — | — | 4·14 |

[a] Measured on a Varian HA-100 instrument; solvent deuteriochloroform and internal standard tetramethylsilane (TMS) if not otherwise stated; chemical shifts in $\delta$(TMS)-scale, splittings in Hz (in parentheses); multiplicity (if given) indicated (if possible) as usually; [b] approximate values from decoupling experiments except for compound *XIII*; [c] analysed as AB or $A_2$ system or subsystem using tickling experiments; [d] in hexadeuteriobenzene; [e] internal standard hexamethyldisiloxane (HMDS), $\delta$(HMDS) = 0·06 p.p.m.; [f] $H_{(10)}$: 2·28 tt (second-order; 10·5, 3·5);

couplings, *i.e.* the fragment $CH_2$—CH(OH)—CH, and hence also the position $C_{(3)}$. This assignment of the position for the OH group is also in agreement with the acylation shifts $\Delta^{(j)}\delta H_i(OH, OTAC) = \delta H_i(C_j—OH) - \delta H_i(C_j—OTAC)$ of trichloroacetylcarbamates obtained *in situ* on reaction of compounds *VIII* and *IX* with trichloroacetylisocyanate, under formation of derivatives *X* and *XI* (TAI-method[12,13];

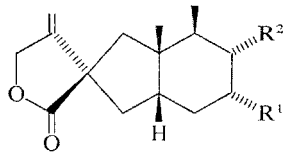
TABLE II
(*Continued*)

| $H_{(12')}$[c] | $H_{(13)}$ | $H_{(13')}$ | $H_{(14)}$ | $H_{(15)}$ |
|---|---|---|---|---|
| 4·73 dt (12·8, 1·9) | 5·10 td (2·2, 0·7) | 5·03 td (1·9, 0·7) | 0·85 (6·5) | 0·99 |
| 4·09 dt (12·8, 1·9) | 4·70 t (2·2) | 4·13 t (1·9) | 0·64 (6·5) | 0·83 |
| 4·75 t (~2) | 5·09 | 5·01 | 0·90 (6·5) | 1·01 |
| 4·78 t (~2) | 5·17[j] | 5·17[j] | 0·88 (6·6) | 1·02 |
| 4·75 t (2·1) | 5·15 td (0·8, 2·3) | 5·01 td (0·8, 2·0) | 0·90 (6·7) | 0·99 |
| 4·74 dt (12·8, 2·15) | 5·12 td (2·2, 0·9) | 5·04 td (2·0, 0·9) | 1·03 (6·4) | 1·02 |
| 4·77 t | 5·23 td | 5·06 | 0·92 (6·6) | 1·02 |
| 4·78 t | 5·10 | 5·10 | 0·99 (6·5) | 1·08 |
| 4·76 t (2) | 5·06 t (2) | 5·06 t (2) | 0·99 (6·5) | 1·21 |
| 4·07 t | 4·50 td | 4·50 td | 0·56 | 0·77 |
| 4·81[r] | 5·10 t (2·1) | 5·10 t (2·1) | 1·02 (6·9) | 0·99 |
| 4·14 | 4·54 | 4·40 | 1·34 | 1·14 |

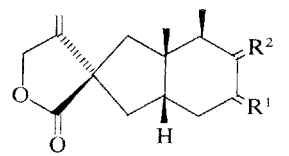
[g] $H_{(2)}$: 5·17 (q, ~3, overlapping with $H_{(13)}$), β-H (O-angeloyl): 6·02qq ; [h] $H_{(3)}$: 5·12 (overlapping with $H_{(13)}$), β-H (O-tigloyl): 6·83; [i] not pure compound, spectrum contains many signals of impurities; [j] overlapping with $H_{(3)}$; [k] $H_{(2)}$: 4·12 (q, 3·1); [l] $H_{(3)}$: 3·42 td (second order, $\Sigma J$ = = 25 Hz); [m] prepared *in situ* by addition of a few drops of trichloroacetyl isocyanate to the deuteriochloroform solution; [n] NH: 8·38 bs; [o] NH: 8·38 bs; [p] $H_{(10)}$: 2·71, $H_{(9)}$: 1·70 t (13·2); [q] $H_{(9)}$: 1·31 t (13·5), $H_{(10)}$: 2·41; [r] AB part with small internal shift, position of the center.

TAC=CONHCOCCl$_3$). While no significant shifts of proton $H_4$ or the methyl protons $H_{14}$ and $H_{15}$ were observed in the case of *X*, in the case of compound *XI* a downfield shift of about 0·30 p.p.m. for the signal of proton $H_4$ was observed (Table II), corresponding to the acylation shift in the position $C_{(3)}$ ($\Delta^{(3)}H_4$), see for example[14-16]. The positions of the hydroxyl groups were further corrobated in the following manner: oxidation of *VIII* gave ketone *XII* the deuteration of which (exchange of four hydrogens in the molecule; determined by mass spectrometry) confirmed the presence of two $CH_2$ groups in the position vicinal to carbonyl. The fragmentation of thioketal *XIV* led to the same conclusion. The fragment $m/e = 145$ in the mass spectrum corresponds to the $CH_3CH{=}CHC(-SCH_2CH_2S^{(+)}=)$ part. Oxidation of compound *IX* gave ketone *XIII* in the $^1$H-NMR spectrum of which the methine proton $H_4$ formed a quartet at 2·59 p.p.m. ($J = 6·8$) in agreement with the position of the carbonyl on $C_{(3)}$.

It is also possible to draw some conclusions regarding the stereochemistry of the substances isolated. In the case of compound *V* the correspondence of its IR, $^1$H-NMR and CD spectra and the mixed melting point with those of authentic bakkenolide A sample indicated an identical structure. A direct correlation of compounds *VI* and *VII* with bakkenolide A *via* thioketals of ketones *XII* and *XIII* could not be carried out. Nevertheless, in view of the simultaneous occurrence of compound *VI*–*VII* in the extracts from the collections from three different localities (of course, chemovars cannot be excluded by this unambiguously) it can be supposed that compounds *VI* and *VII* will also have the same absolute configuration on the centres $C_{(4)}$, $C_{(5)}$, $C_{(7)}$ and $C_{(10)}$. This biogenetic aspect is also strengthened by the relationship with substances of the eremophilane type for which an absolute configuration β at 4-$CH_3$, 5-$CH_3$ and $H_{(10)}$ is typical, especially in substances from the *Petasites* genus. All substances of the bakkenolide type isolated so far also had this configuration. In addition to this they also had the same configuration at the $C_{(7)}$ centre.




*V*, $R^1 = R^2 = H$
*VI*, $R^1$ = Angeloyloxy, $R^2 = H$
*VII*, $R^1 = H$, $R^2$ = Tigloyloxy
*VIII*, $R^1 = OH$, $R^2 = H$
*IX*, $R^1 = H$, $R^2 = OH$
*X*, $R^1 = OCONHCOCCl_3$, $R^2 = H$
*XI*, $R^1 = H$, $R^2 = OCONHCOCCl_3$




*XII*, $R^1 = O$, $R^2 = H_2$
*XIII*, $R^1 = H_2$, $R^2 = O$
*XIV*, $R^1 = S(CH_2)_2S$, $R^2 = H_2$

An identical configuration of compounds *V*–*VII* is also indicated by the agreement of the characteristic parameters of the $^1$H-NMR spectra in Table II, demonstrated in Table III by the differences of the chemical shifts $\Delta^{(j)}\delta H_i(H, R) = H_i(R_j = H) - H_i(R_j)$ of protons of bakkenolide A (*V*) and its derivatives *VIII*–*XI* ($R_j$ = OH, OTAC; $j$ = 2·3), as well as by the acylation shifts $\Delta^{(j)}\delta H_i$(OH, OTAC). From Table III it is evident that the substituent in the position $C_{(2)}$ induces significant variation (the changes about 0·1 p.p.m. and more are considered as such) in the chemical shift $H_{(4)}$ and $H_{(13)}$ only, while in the position $C_{(3)}$ only in protons $H_{(4)}$ and $H_{(14)}$. Such a small variability of the chemical shifts can be interpreted under the supposition that the observed differences in the chemical shifts represent long-range shielding effects of substituents, *i.e.* that the configuration and the conformation of the skeleton in substances *V*, *VIII*–*XI* are equal. Under this supposition, and when the paramagnetic shift of $H_{(15)}$ is significant, it follows that the probable configuration of $H_{(4)}$ and 2-OR groups is diaxial, and from the existence of the long-range effect on $H_{(13)}$ it follows that the $H_{(13)}$ proton is close to 2-OR, which can be expected only if the rings A and B are *cis* annelated and the exomethylene group is in *endo*-configuration with respect to the ring A. From these aspects it follows that the relative configuration of $H_{(10)}$, $C_{(5)}$—$CH_3$ and $C_{(4)}$—$CH_3$ is β, and of the $C_{(7)}$—$C_{(11)}$ bond is α, and further follows a chair conformation of the A ring with an axial substituent on $C_{(2)}$ and an equatorial substituent on $C_{(3)}$. The equatorial ori-

Table III

Comparison of Long-range Shielding Effects of the Substituents R = OH, OTAC (TAC = = $CONHCOCCl_3$) in Positions $j$ = 2, 3 of the Bakkenolide Skeleton $\Delta^{(j)}\delta H_i(H, R) = \delta H_i(R_j = H) - \delta H_i(R_j)$ and of Acylation Shifts (*in situ*) $\Delta^{(j)}H_i(OH, OTAC) = \delta H_i(C_j—OH) - \delta H_i(C_j—OTAC)$

| $H_{(i)}$ [a] | $H_{(4)}$ [b] | $H_{(6)}$ | $H_{(6')}$ | $H_{(12)}$ | $H_{(12')}$ | $H_{(13)}$ | $H_{(13')}$ | $H_{(14)}$ | $H_{(15)}$ |
|---|---|---|---|---|---|---|---|---|---|
| $\Delta^2\delta H_i$(H, OH) | −0·58 | −0·07 | +0·01 | +0·03 | −0·02 | −0·05 | +0·02 | −0·05 | 0·00 |
| $\Delta^2\delta H_i$(H, OTAC) | −0·59 | −0·05 | −0·05 | +0·01 | −0·04 | −0·13 | −0·03 | −0·07 | −0·03 |
| $\Delta^3\delta H_i$(H, OH) | −0·10 | −0·01 | −0·01 | −0·01 | −0·01 | −0·02 | −0·01 | −0·18 | −0·03 |
| $\Delta^3\delta H_i$(H, OTAC) | −0·40 | −0·05 | −0·05 | 0·00 | −0·05 | 0·00 | −0·07 | −0·14 | −0·09 |
| $\Delta^2\delta H_i$(OH, OTAC) | −0·01 | +0·02 | −0·06 | −0·02 | −0·02 | −0·08 | −0·05 | −0·02 | −0·03 |
| $\Delta^3\delta H_i$(OH, OTAC) | −0·30 | −0·04 | −0·04 | +0·01 | −0·04 | +0·02 | −0·06 | +0·04 | −0·06 |

[a] Calculated from Table II, entries *V*, *VIII*, *IX*, *X*, *XI* (in deuteriochloroform); [b] inaccurate shift differences because of approximate positions of signals of $H_{(4)}$ estimated solely by a single decoupling experiment; in view of the width of the multiplet of $H_{(4)}$ about 40 Hz it is impossible to define the character of 1,3-interaction between $H_{(4)}$ and OH in position $C_{(2)}$.

entation of the 3-OH group also follows from the splitting constants of the multiplet of $H_{(3)}$ : $J_{3,4} \cong J_{2,3} \cong 10$ Hz $= J_{a,a}$ and $J_{2',3} \cong 4$ Hz $= j_{e,a}$ and the axial conformation of the 2-OR group follows from the splitting constants of the multiplet for $H_{(2)}$ : $J_a \cong J_2 \cong J_3 \cong J_4 \cong 3{\cdot}1$ Hz. These aspects of the $^1$H-NMR spectrum lead therefore to the same conclusions both on the configuration and on conformation of substance *V*, following from its identity with bakkenolide A. The normal steroidal chair-conformation of the ring A also followed directly from the $^1$H-NMR spectrum of bakkenolide A and it was also determined by X-ray analysis of 1,9-dihydroxy derivatives[17].

As follows from the identity of the CE of the lactone chromophore on the CD spectra of *V*, *VIII*, *IX*, *XII* and *XIII*, the absolute configuration of the $C_{(7)}$ centre is in all these substances probably identical.

The components of bakkenolide type found in *Homogyne alpina* (L.) CASS. are so far known in *Petasites japonicus* (SIEB. et ZUC.) MAXIM. subsp. *giganteus* KITAM.[6–11], in *Petasites albus* (L.) GAERTN.[18], and in *Ligularia hodgsonii* HOOK. f.[19]. The identity of the discovered substances of this type, which are biogenetically related to the substances of eremophilane type, fully agrees with the classification of the species *Homogyne alpina* into the subtribe *Senecioneae* where the *Petasites* and *Ligularia* genera also belong.

## EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The IR spectra were measured on a C. Zeiss UR-20 instrument, the UV spectra on an Optica Milano CF 4 spectrophotometer, in ethanol, and the CD spectra on a Roussel-Jouan Dichrographe CD-185 apparatus, in methanol. The $^1$H-NMR spectra were determined on a Varian HA 100 instrument under the conditions given in Tables I and II. The molecular weights were determined by mass spectrometry on a AEI MS 902 instrument. For thin-layer chromatography Kieselgel G nach Stahl (Merck) was used and for column chromatography silica gel according to Pitra[20] (Service laboratories in Lysolaje), prepared by sedimentation procedure, dried and deactivated with 12% of water, and Silpearl (Kavalier, Votice) treated in the same manner. The material for the isolation of substances was collected in North Bohemia near Rudolfov in the Jizera Mountains in summer 1966 (orienting collection), then in summer 1967 at the same site (first collection) and near Malá Úpa in the Giant Mountains in autumn 1970 (second collection).

### Isolation of Substances

The dried and ground plant material (about 3 kg) was extracted with light petroleum at room temperature. The extract (37 g) after evaporation of solvents at reduced pressure was separated chromatographically on a silica gel column (1 kg). A mixture of light petroleum–benzene was used for stepwise gradient elution (100 : 0, 100 : 1, 50 : 1, 10 : 1, 1 : 1, 0 : 100), and further benzene–ether–ethanol (100 : 0 : 0, 100 : 5 : 0, 100 : 5 : 2). This was done so that the more polar component of the eluent was added to the distillate of each fraction in the given ratio. Fractions of 1 000 ml were collected which were evaporated and combined according to the content of cor-

responding substances (monitored by thin-layer chromatography), giving the following fractions of the extract (eluent, eluent volume in ml, dry weight of the residue in g): $E_1$, light petroleum, 10000, 10·6; $E_2$, light petroleum–benzene, 1000, 3·0; $E_3$, benzene, 1000, 3·2; $E_4$, benzene, 2000, 7·4; $E_5$, benzene–ether, 2000, 2·3; $E_6$, benzene–ether, 5000, 4·2; $E_7$, benzene–ether, 2000, 3·9; $E_8$, benzene–ether–ethanol, 4000, 2·4. The extract obtained from the second collection (65 g) was worked up in the same manner and a proportionally larger amount of fractions $E_1$ to $E_8$ was obtained. A qualitative difference was observed in fraction $E_6$ only, where the relative content of single bakkenolide esters was different (see text below). The material from the orienting collection was also worked up in the given manner, but roots and leaves were worked up separately. The ratio between the content of the main components in the roots and the leaves, determined from the weights of the chromatographic fractions of the extract, was about 4 : 3 in euparins, and 5 : 2 in bakkenolides.

Euparin (I)

From fraction $E_3$ a yellow substance (0·8 g) crystallized out from benzene, which after crystallization from ethanol had m.p. 122–123°C. IR spectrum: 1643, 1602, 1662, 2600–3300 $cm^{-1}$; UV spectrum: $\lambda_{max_1}$ 262 nm (log $\varepsilon$ 3·59), $\lambda_{max_2}$ 290 nm (log $\varepsilon$ 3·19), $\lambda_{max_3}$ 359 nm (log $\varepsilon$ 2·79); mass spectrum: *M/e* 216, *m/e* 201, 173, 115, 91. For $C_{13}H_{12}O_3$ (216·2) calculated: 72·21% C, 5·59% H; found: 72·15% C, 5·59% H.

7-Methoxyeuparin (*II*)

Fraction $E_5$ was rechromatographed on a silica gel column with light petroleum–dichloromethane mixture (2 : 1). From the combined purest fractions a residue (2 g) was obtained which was crystallized from light petroleum (b.p. 60–80°C) to give crystals melting at 95–96°C. IR spectrum: 1564, 1640, ~3000 $cm^{-1}$; UV spectrum: $\lambda_{max_1}$ 237 (log $\varepsilon$ 3·64), $\lambda_{max_2}$ 270 (log $\varepsilon$ 4·11), $\lambda_{max_3}$ 363 (log $\varepsilon$ 3·15). Mass spectrum: *M/e* 246, *m/e* 228, 213, 203, 201. For $C_{14}H_{14}O_4$ (246·3) calculated: 68·28% C, 5·73% H; found: 68·79% C, 5·97% H.

Tetrahydro Derivatives *III* and *IV*

Euparin (*I*, 50 mg) was hydrogenated at room temperature and atmospheric pressure in ethyl acetate (10 ml), using 5% $Pd/SrCO_3$ (25 mg) as catalyst. When hydrogen absorption ceased the product was purified chromatographically on silica gel using light petroleum–ether (20 : 1) for elution. Tetrahydro derivative *III* was obtained as the main product. Mass spectrum: *M/e* 220, *m/e* 205, 177, 165, 149.

In a similar manner 7-methoxyeuparin (*II*, 50 mg) was hydrogenated to give tetrahydro derivative *IV*. Mass spectrum for $C_{14}H_{18}O_4$ (250·1205) found *M/e* 250·1202; *m/e* 235, 195.

Bakkenolide A (*V*)

From fraction $E_4$ dissolved in benzene a substance (5·3 g) crystallized out which after recrystalisation from benzene melted at 80–81°C. From the mother liquors, which were purified chromatographically on silica gel with benzene as eluent, a further 5·1 g of substance were obtained, $[\alpha]_D$ +18·7° (*c* 0·52, methanol), IR spectrum: 1781, 1671, 3080, 896 $cm^{-1}$; UV spectrum: $\lambda_{max}$ 212 nm (log $\varepsilon$ 3·01); CD spectrum: $\Delta\varepsilon_{210}$ −2·11 (the sample from N. Abe had $\Delta\varepsilon_{210}$ −2·28); Mass spectrum: *M/e* 234; *m/e* 219, 189, 124, 111, 109. For $C_{15}H_{22}O_2$ (234·3) calculated: 76·88% C, 9·46% H; found: 77·00% C, 9·41% H.

Angeloyl Ester of 2-Hydroxybakkenolide A (*VI*) and Tigloyl Ester of 3-Hydroxybakkenolide A (*VII*)

From collective fraction $E_6$ the fraction of bakkenolide esters was isolated by chromatography on silica gel, using benzene–ether 10 : 1 for elution. These could be separated by multiple thin-layer chromatography in light petroleum–ether 5 : 1 to two substances with very close $R_F$ values.

On chromatography on a hundred-fold amount of silica gel, using light petroleum–ether 10 : 1 mixture as eluent, this ester fraction was separated so that from its first part substance *VI* crystallized out, m.p. 62–65°C; IR spectrum: 1780, 1714, 1671, 1640 and 895 $cm^{-1}$; CD spectrum: $\Delta\varepsilon_{220}$ −2·51, $\Delta\varepsilon_{225}$ +0·2; mass spectrum: *M/e* 332, *m/e* 232, 122, 111, 107. For $C_{20}H_{28}O_4$ (332·4) calculated: 72·26% C, 8·49% H; found: 71·50% C, 8·96% H.

From the second part after repeated chromatography the non-crystalline substance *VII* was obtained in a high purity (according to $^1$H-NMR spectrum). IR spectrum: 1780, 1709, 1672, 1651 and 896 $cm^{-1}$; mass spectrum: *M/e* 332, *m/e* 232, 122, 83, 55.

Fraction $E_6$ from the second collection was worked up in the same manner, but even after repeated chromatographic purification compounds *VI* and *VII* could not be obtained in crystalline state. Two chromatographically pure fractions were obtained only. A and B, the main components of which were substances *VI* and *VII*. As impurities esters of 2-hydroxy and 3-hydroxy bakkenolide A were present as evident from the analyses of the products after hydrolysis (see below). The proportion of the esters could be changed considerably in favour of the main components by repeated chromatographic separations. The purity of non-crystalline substances and various enriched fractions A and B was checked by gas chromatography.

2-Hydroxybakkenolide A (*VIII*) and 3-Hydroxybakkenolide A (*IX*)

Ethanol (25 ml) was added into a 20% NaOH solution (50 ml) located in a closed apparatus in which air was substituted by nitrogen, and a solution of ester *VI* (400 mg) in ethanol (25 ml) was added dropwise to it under stirring and heating. The mixture was then refluxed under nitrogen for one hour. Water was added (20 ml) and a part of water and ethanol (50 ml) distilled off. The mixture was acidified with 1M-$H_2SO_4$ to pH 4 and extracted with ether. The extract was washed with a $NaHCO_3$ solution, dried and evaporated. The residue was purified chromatographically on a silica gel column with benzene–ether (20 : 1), giving pure hydroxy derivative *VIII*, m.p. 91–93°C; IR spectrum: 1768, 1673, 3093, 3615 and 3515 $cm^{-1}$; CD spectrum: $\Delta\varepsilon_{210}$ −1·82; mass spectrum: *M/e* 250, *m/e* 232, 217, 122, 107, 91. For $C_{15}H_{22}O_3$ (250·3); calculated: 71·97% C, 8·86% H; found: 72·04% C, 8·74% H.

In the same manner ester *VII* was hydrolysed and after chromatographic purification of the product hydroxy derivative *IX* was obtained melting at 97–100°C; IR spectrum: 1770, 1675, 3090, 3625, 3610 and 3530 $cm^{-1}$; CD spectrum: $\Delta\varepsilon_{210}$ −1·59; mass spectrum: *M/e* 250, 232, 122.

Fraction A and fraction B, obtained from fraction $E_6$ from the second collection (see the preceding section) were submitted to alkaline hydrolysis under the same conditions as above. From fraction A two chromatographically separable crystalline derivatives were obtained in a 5 : 1 ratio. From fraction B also two crystalline derivatives were obtained in a 1 : 3 ratio. According to melting points and $^1$H-NMR spectra the main product from fraction A is identical with the by-product from fraction B, and also with 2-hydroxybakkenolide A (*VIII*). Further, the main product from fraction B is identical with the by-product from fraction A, as well as with 3-hydroxybakkenolide A (*IX*). From this it follows that in fraction $E_6$ all combinations of angelyl- and tiglyl esters of alcohols *VIII* and *IX* are present in various ratios (depending on the locality or the time of collection), and that they are rather separated according to the type of the ester group

than according to the position of the substituent. The esters, which occur as impurities only, were not isolated. In subsequent work single esters were not separated, but the whole fraction $E_6$ was hydrolysed directly and separated afterwards, which considerably simplified the obtaining of hydroxy derivatives *VIII* and *IX*.

2-Oxobakkenolide A (*XII*) and 3-Oxobakkenolide A (*XIII*)

Jones's oxidation reagent was added dropwise and under stirring into a solution of hydroxy derivative *VIII* (100 mg) in acetone (10 ml) until the colour of the reaction mixture was persistently brown. The course of the reaction was followed using thin-layer chromatography. After three hours water (25 ml) was added and part of the acetone and water was distilled off. The remaining mixture was extracted with ether, the extract was dried and evaporated, and the residue was purified chromatographically on a silica gel column with benzene–ether (20 : 1) as eluent. A syrup was obtained which had the following characteristics: IR spectrum: 1772, 1152, 1713, 1673 $cm^{-1}$; CD spectrum: $\Delta\varepsilon_{210}$ −2·14, $\Delta\varepsilon_{290}$ +1·34; mass spectrum: for $C_{15}H_{20}O_3$ (248·1412) found *M/e* 248·1410, *m/e* 233, 138, 123, 111, 96, 91.

In the same manner hydroxy derivative IX was oxidized. After chromatographic purification of the product oxo derivative *XIII* was obtained with m.p. 78–81°C. IR spectrum: 1776, 1149, 1711, 1673 $cm^{-1}$; CD spectrum: $\Delta\varepsilon_{210}$ −0·62, $\Delta\varepsilon_{290}$ −1·75; mass spectrum: *M/e*: 248, *m/e*: 230, 177, 138, 123.

Deuteration of 2-Oxobakkenolide A (*XII*)

Sodium (20 mg) was dissolved in a mixture of $D_2O$ (99·5%, 2·5 ml) and dioxan (5 ml) and ketone *XII* (25 mg) dissolved in dioxan (5 ml) was added dropwise to the solution under stirring and heating. Before the start of the reaction air was expelled by nitrogen from the apparatus, and the mixture was refluxed under nitrogen for 30 minutes and evaporated. Dioxan (10 ml) and $D_2O$ (3 ml) were then added to the residue and the mixture was refluxed for another 20 minutes. After acidification with 1M-$H_2SO_4$ (in $D_2O$) and extraction with ether the extract was dried and evaporated. The residual product was purified on a short silica gel column (deactivated with 13% of $D_2O$), using benzene–ether 20 : 1 for elution. According to the mass spectrum deuteration took place up to the fourth stage.

2-Oxobakkenolide A Ethylenedithio Ketal (*XIV*)

A mixture of ketone *XII* (25 ml), acetic acid (4 ml), ethanedithiol (0·25 ml) and $BF_3$-etherate (0·25 ml) was allowed to stand at room temperature for 20 hours. After dilution with water (20 ml) and addition of KOH (6 g) solution in water (12 ml) under cooling with ice the product was worked up by extraction with ether, drying the extract and evaporation of the solvent. The residue was purified chromatographically on a silica gel column with benzene. The non-crystalline ethylenedithio ketal *XIV* obtained contained in its mass spectrum a peak of *M/e* 324·121147, corresponding to the molecule $C_{17}H_{24}O_2S_2$ and a fragment of *m/e* 145·014346, corresponding to the composition $C_6H_9S_2$.

*The elemental analyses were carried out in the analytical department of this Institute. Gas Chromatographies and the records of orienting* IR *spectra were carried out by Mrs S. Holubová, under the direction of Dr V. Lukeš. The* IR, UV *ans* CD *spectra were measured by Mr P. Formánek and interpreted by Dr S. Vašičková, the mass spectra were measured by Mrs M. Vokáčová and*

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*Note added in proof:* For the numbering of the bakkenolide skeleton see refs 8—11.