249. Structure and Stereochemistry of New Sesquiterpene Esters from *Echinacea purpurea* (L.) MOENCH

(Preliminary communication)

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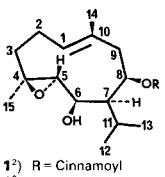
(10.IX.85)

The structure elucidation of four new constituents from the roots of *Echinacea purpurea* is described. They are shown to be cinnamoyl esters of sesquiterpene alcohols with a germacrane or a guaiane skeleton. First pharmacological results indicate immunological activities.

Introduction. – Extracts of *Echinacea purpurea* leaves and roots have a long tradition in therapy for wound healing and for the unspecific enhancement of the immune system [1]. *Wagner* and *Proksch* [2] recently published the isolation of two polysaccharides with immunostimulatory activities. Now we report the isolation and structure determination of the first nonvolatile sesquiterpene constituents of *Echinacea:* cinnamoyl-echinadiol (= 7,8-epoxy-9-hydroxy-10-isopropyl-3,7-dimethyl-3-cyclodecen-1-yl cinnamate; **1**), cinnamoyl-epoxyechinadiol (= 4,5:8,9-diepoxy-3-hydroxy-2-isopropyl-5,9-dimethyl-cyclodecyl cinnamate; **3**), cinnamoyl-echinaxanthol (= 5-hydroxy-4-isopropyl-1-methyl-7-(3-oxobutyl)bicyclo[4.1.0]hept-3-yl cinnamate; **6**), and cinnamoyl-dihydroxynardol (2,10-dihydroxy-3-isopropyl-10-methyl-6-methylidenebicyclo[5.3.0]dec-4-yl cinnamate; **8**). They were obtained, each in about 0.1% yield, from the $CHCl_3$ extract of the roots of



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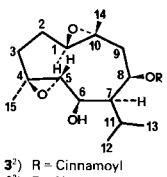
2[2]) R = H



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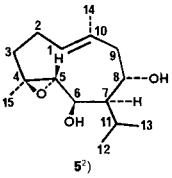

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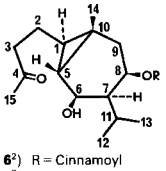
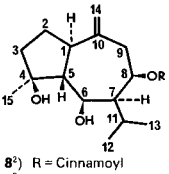


6[2]) R = Cinnamoyl
7[2]) R = H



8[2]) R = Cinnamoyl
9[2]) R = H

Echinacea purpurea by silica gel column chromatography and successive prep. TLC and HPLC. Hydrolysis of the oily cinnamoyl esters with NaOH (10% MeOH solution) gave, beside cinnamic acid, the sesquiterpene alcohols echinadiol (= 9,10-epoxy-2-isopropyl-5,9-dimethyl-5-cyclodecene-1,3-diol; **2**), epoxyechinadiol (= 4,5:8,9-diepoxy-2-isopropyl-5,9-dimethylcyclodecane-1,3-diol; **4**), echinaxanthol (= 2-(3,5-dihydroxy-4-isopropyl-1-methylbicyclo[4.1.0]hept-7-yl)ethyl methyl ketone; **7**), and dihydroxynardol (= 3-isopropyl-10-methyl-6-methylidenebicyclo[5.3.0]decan-2,4,10-triol; **9**) in pure form.

Echinadiol (**2**) *and Epoxyechinadiol* (**4**). The conformation of **2** and **4** was determined by X-ray structure analysis. Both compounds crystallized from toluene/$CHCl_3$ in colourless plates[1]).

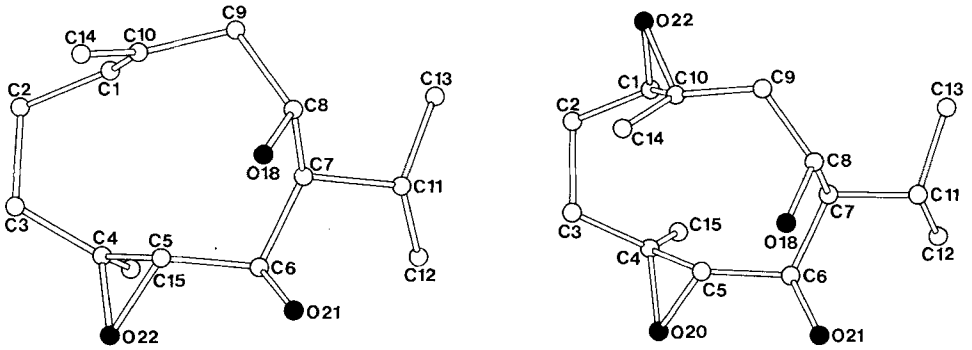


Fig. *X-Ray structure of echinadiol* (**2**) *and epoxyechinadiol* (**4**)[2])

The crystal structures are shown in the *Figure*. Both compounds possess a germacrane skeleton, a 4,5-epoxy group, and OH groups at C(6) and C(8)[2]). Compound **2** has a double bond at C(1), C(10), which is replaced in **4** by a second epoxy group.

A compound similar to echinadiol (**2**) named shiromodiol (**5**) was isolated from the *Lauracee Parabenzoin trilobum* [3] and recently from the *Apiacee Thapsia villosa* [4].

Table 1. *Comparison of Torsion Angles*

	Echinadiol (**2**)	Epoxyechinadiol (**4**)	Shiromodiol (**5**)
C(8)–C(9)–C(10)–C(1)	–90.4	–85.1	64.0
C(10)–C(1)–C(2)–C(3)	–73.0	–70.4	111.9
C(2)–C(3)–C(4)–C(5)	91.2	94.5	154.3
C(6)–C(7)–C(8)–O(18)	13.9	14.4	108.7
C(11)–C(7)–C(8)–O(18)	–112.8	–111.7	–21.5
O(18)–C(8)–C(9)–C(10)	–58.9	–58.2	174.7

[1]) Crystal data of **2**: $a = 8.413$, $b = 10.962$, $c = 16.492$ Å; space group orthorhombic $P2_12_12_1$, $Z = 4$, $d_{meas.} = 1.14$ g/cm^3; 1162 unique reflections were measured (observed $1081 \geq 3\sigma(I)$) on a *Nicolet-R3m* diffractometer with Cu*K* radiation, Ni-filtered. Crystal data of **4**: $a = 8.269$, $b = 10.968$, $c = 16.700$ Å; space group orthorhombic $P2_12_11_1$, $Z = 4$, $d_{meas.} = 1.10$ g/cm^3; 1159 unique reflections were measured (observed $1091 \geq 3\sigma(I)$).

The two structures were solved by direct methods using SHEXTL programmes. All atoms excluding H-atoms were found in *E* maps followed by difference *Fourier* maps. The refinements converged at $R = 9.17$ and 9.35%, respectively.

[2]) Arbitrary numbering; systematic names, see *Introduction*.

Comparison of the X-ray data of **5** [5] and **2** showed that there are different torsion angles for the orientation of OH-C(8) and the double bond as shown in *Table 1*. When H-C(7) of **2** is considered to be in an α-position, both OH groups appear in the β-orientation, whereas OH-C(8) of **5** has the α-orientation. The different torsion angles for the *trans*-double bond in **2** indicate inflection of this bond. The relative geometries of the common asymmetric centres of **2** and **4** are the same, as are the relative dispositions of the two methyl groups C(14) and C(15) which appear in an *'anti'* arrangement. *Bohlman et al.* [6] have isolated an esterified germacranediol with the same constitution as **4** but with the β-orientation of CH_3(15). Also recently, the 1,10-epoxy derivative of shiromodiol with a conformation ${}_{15}D^5,{}^1D_{14}$ has been found [4]. Hence, epoxyechinadiol (**4**) is a new sesquiter-

Table 2. *^{1}H and ^{13}C-NMR Data for Compounds* **7** *and* **9** *in $CDCl_3$*[2])

	7		
	^{13}C-NMR	^{1}H-NMR	
	δ[ppm]	δ[ppm]	J[Hz]
CH(1)	28.8 (*d*)	0.23	$J(1,2A) = 7.3$
CH_2(2)	23.9 (*t*)	1.65 (H_A), 1.59 (H_B)	$J(1,2B) = 7.3$
CH_2(3)	43.8 (*t*)	2.51	$J(1,5) = 5.5$
C(4)	208.8 (*s*)	–	$J(2A, 2B) = 14.4$
CH(5)	35.1 (*d*)	0.83	$J(2A,3) = 7.5$
CH(6)	67.0 (*d*)	4.26	$J(2B,3) = 7.5$
CH(7)	45.3 (*d*)	0.48	$J(5,6) = 1.7$
CH(8)	67.0 (*d*)	4.02	$J(6,7) = J(7,8) = 1.3$
CH_2(9)	43.3 (*t*)	2.01 (H_A), 1.70 (H_B)	$J(7,11) = 10.4$
C(10)	16.0 (*s*)	–	$J(8,9A) = 2.4$
CH(11)	24.6 (*d*)	2.09	$J(8,9B) = 3.7$
CH_3(12)	21.2 (*q*)	1.00	$J(9A, 9B) = -15.2$
CH_3(13)	20.6 (*q*)	0.96	$J(11,12) = 6.6$
CH_3(14) or CH_2(14)	22.7 (*q*)	1.17	$J(11,13) = 6.6$
CH_3(15)	30.0 (*q*)	2.16	
	9		
	^{13}C-NMR	^{1}H-NMR	
	δ[ppm]	δ[ppm]	J[Hz]
CH(1)	43.4 (*d*)	2.31[a])	$J(5,6) = 8.9$
CH_2(2)	28.5 (*t*)	1.78–1.64	$J(6,7) = 3.8$
CH_2(3)	40.2 (*t*)	1.86 (H_A), 1.78–1.64 (H_B)	$J(7,8) = 1.4$
C(4)	80.3 (*s*)	–	$J(7,11) = 9.7$
CH(5)	59.6 (*d*)	2.31[a])	$J(8,9A) = 5.6$
CH(6)	71.7 (*d*)	4.13	$J(8,9B) = 7.4$
CH(7)	50.1 (*d*)	1.10	$J(9A,9B) = -14.2$
CH(8)	71.3 (*d*)	4.29	$J(9A,14) = 1$
CH_2(9)	44.7 (*t*)	2.64 (H_A), 2.60 (H_B)	$J(9B,14) = 0.7$
C(10)	149.2 (*s*)	–	$J(11,12) = 6.6$
CH(11)	26.5 (*d*)	2.10	$J(11,13) = 6.6$
CH_3(12)	21.5 (*q*)	1.06	
CH_3(13)	21.6 (*q*)	0.99	
CH_3(14) or CH_2(14)	111.1 (*t*)	4.76 (H_A), 4.75 (H_B)	
CH_3(15)	24.2 (*q*)	1.25	

[a]) In C_6D_6, C*H*(1) at 2.07 and C*H*(5) at 2.42 ppm; $J(1,5) = 12.9$ and $J(5,6) = 9.4$ Hz.

pene epoxy alcohol. As the signal of H-C(8) in the ^{1}H-NMR spectra of the cinnamates **1** and **3** is shifted downfield by 1.2 ppm compared to those in the spectra of the alcohols **2** and **4**, the linkage to the cinnamic-acid moieties must be at C(8).

Echinaxanthol (**7**) *and Dihydroxynardol* (**9**). The structures of these two alcohols were determined in solution as they could not be obtained in crystalline form. Both compounds exhibited molecular ions (M^+) at 254, indicating that they were isomeric with **2** and compatible with the molecular formula $C_{15}H_{26}O_3$. In each case, fragment ions at *m/z* 236 and 218 produced by successive loss of H_2O from M^+ suggested the presence of at least two OH groups. The number and nature of each group in the molecules were obtained from the ^{1}H- and ^{13}C-NMR data given in *Table 2*. The final structures of **7** and **9** were deduced from 2D ^{1}H-NMR homonuclear shift correlated (COSY) spectra and extensive ^{1}H-NMR homonuclear NOE difference experiments. Compound **7** is a cyclopropanoid secoguaiane derivative. Although *Wada et al.* [7] isolated a similar shiromodiol-derived compound after thermal degradation of shiromodiol, the sesquiterpenediol **7** is not artificial but a natural constituent of *E. purpurea*. Compound **9** appears to be the 6,8-dihydroxy derivative of nardol, which has previously been isolated from *Nardostachys jatamansi* [8].

The observations of cross peaks in the COSY spectrum of **7** in $CDCl_3$ allowed the entire fragment C(3) to C(9) *via* C(1) and C(5) to be deduced. The shifts of C*H*(1) and C*H*(5) showed the presence of the cyclopropane-ring system, while the ^{1}H and ^{13}C shifts indicated the position of the methyl-ketone moiety (IR: 1710 cm^{-1}). The magnitude of the vicinal coupling constants (*Table 2*) and NOE difference spectra unambiguously determined the position of the remaining CH_3 group and the relative configuration of the various substituents.

Similarly, the COSY spectrum of **9** in $CDCl_3$, together with an ^{1}H-NMR spectrum in C_6D_6, homonuclear coupling, and spectral simulations, gave the partial fragment C(3) to C(14) *via* C(1), C(5), and C(10), and a small second fragment C(CH_3)OH. Of the various possible combinations of these two fragments, only the proposed structure with the relative geometries shown was compatible with the W coupling between C*H*(6) and C*H*(8) and the large vicinal *trans* couplings between C*H*(1) and C*H*(5), and C*H*(5) and C*H*(6). Likewise, the NOE data, in particular the 'across-the-ring' interactions C*H*(1)···CH_3(15), C*H*(1)···C*H*(7), C*H*(5)···C*H*(9), and C*H*(5)···C*H*(2)/C*H*(3) were indicative of **9**.

The ester linkages for **6** and **8** could again be unambiguously shown to be at C(8).

Immunological activity tests of these new sesquiterpene esters showed that they enhanced granulocyte *phagocytosis in vitro* up to 30%, and **1**, in particular, showed significant activity in the lymphocyte transformation test. It is likely that these esters contribute to the immunostimulating activity of the *Echinacea purpurea* drug. Detailed chemical and experimental data and pharmacological results will be published in due course.

We thank Dr. *O. Seligmann* for several ^{1}H-NMR spectra, Miss *S. Streicher* for technical assistance and the *BMFT* (Jülich) for financial support.

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