ISOLATION AND STRUCTURE OF 3-EPINOBILIN, 1,10-EPOXYNOBILIN AND 3-DEHYDRONOBILIN — OTHER SESQUITERPENIC LACTONES FROM THE FLOWERS OF *Anthemis nobilis* L. REVISION OF THE STRUCTURE OF NOBILIN AND EUCANNABINOLIDE*

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From the lactone fraction of the flowers of *Anthemis nobilis* L. 3-epinobilin (*II*), 1,10-epoxynobilin (*III*) and 3-dehydronobilin (*IV*) were isolated in addition to nobilin (*I*). On the basis of physical methods, especially ^{1}H-NMR spectroscopy the structure of nobilin proposed by us earlier was revised to *I*, and for the other mentioned sesquiterpenic lactones the structures *II* to *IV* were derived.

During a large scale isolation of nobilin by the method described[1] we have detected the presence of other substances in crude nobilin fraction by thin layer chromatography on silica gel in addition to nobilin (*I*). On repeated chromatography on a silica gel column we succeeded in isolating relatively large amounts of nobilin (*I*) in addi-
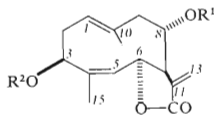
I, R^1 = COC(CH$_3$)=CHCH$_3$; R^2 = H
XXVII, R^1 = COC(CH$_3$)=CHCH$_3$; R^2 = COCH$_3$
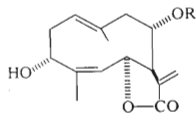
II, R = COC(CH$_3$)=CHCH$_3$
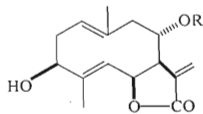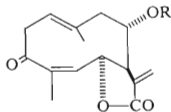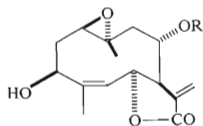
III, R = COC(CH$_3$)=CHCH$_3$ *IV*, R = COC(CH$_3$)=CHCH$_3$ *V*, R = COC(CH$_3$)=CHCH$_3$

tion to minor components which are closely related with nobilin (I) according to their structure, derived predominantly by ^{1}H-NMR spectroscopy and which we named after the elucidation of their structures 3-epinobilin (II), 1,10-epoxynobilin (III) and 3-dehydronobilin (IV).

In connection with the study of the structures of these new lactones we reconsidered the structure of nobilin for which we proposed formula V some time ago. According to the empirical rule[3] that $J_{7,13}$ (cis-lactone) $\leqq 3$ Hz, cis-annelation of the lactone ring with a ten-membered ring has been proposed for nobilin, as in the case of eucannabinolide[4] (VI). It has been shown later by Herz and Wahlberg in connection with the determination of the structure of provincialin[5] (VII) that the mentioned empirical rule[3] does not apply for germacranolides with the $\Delta^{1(10)}$-$trans$ and $\Delta^{4(5)}$-cis configuration of the double bonds (heliangolides[6]), as well as in some other instances. On the basis of the identity of the characteristic ^{1}H-NMR parameters of the sesquiterpenic nucleus of provincialin (VII) and eucannabinolide (VI), found by Herz and Wahlberg[5], the structure expressed by formula $VIII$ must be assigned quite clearly to eucannabinolide. From a detailed analysis of the validity of stereochemical implications of the annelation of the γ-lactone ring, based on vicinal interactions $J_{7,7\pm1}$ and allylic interactions $J_{7,13}$ of the proton H_7 in standard sesquiterpenic ($C_{(7)}$—$C_{(7\pm1)}$) lactones (the numbering of the carbon atoms of the sesquiterpenic skeleton is conventional) it follows that the dihedral angles of the bonds on the fragment $C_{(7)}$—$C_{(7\pm1)}$ and the allylic angles $\measuredangle$ H_7—$C_{(7)}$—$C_{(11)}$—$C_{(13)}$ are not always mutually unambiguously correlated with respect to the formal assignment of the relative configuration of, for example, protons H_7 and $H_{7\pm1}$. The determining factor is the value and the conformation of the annelated cycle limiting the dihedral angle $\Phi_R = \measuredangle$ $C_{(7\pm2)}$—$C_{(7\pm1)}$—$C_{(7)}$—$C_{(7\pm1)}$. The correlation is important for $trans$ lactones, for which the values $|J_{7,7\pm1}| > 10$ and $|J_{7,13}| > 3$ should be typical in the case of conformations with $\Phi < 120°$, and the values $|J_{7,7\pm1}| < 3$ and $|J_{7,13}| < 3$ in the case of conformation with $\Phi > 120°C$ (revised lactone rule)[7]. In germacranolides the endocyclic double bonds represent direct restrictive factors, limiting conformational possibilities of the ten-membered carbon cycle, and, hence, the limitation of Φ_R may be expected in the first place in dependence on the relative positions of all restrictive factors, $i.e.$ both of the double bonds and of the γ-lactone ring. Typical examples are germacra-1(10),4-dien-6,12-olides and their 1,10-epoxy derivatives for which the possibilities of the realization of optimum conformations are dependent directly on the configuration of the double bond between $C_{(4)}$ and $C_{(5)}$. While for germacra-1(10)-$trans$-4(5)-cis-dien-6,12-olides the conformation with $\Phi < 120°$ should be typical, the conformation with $\Phi > 120°$ is typical for the 4(5)-cis isomers. In these cases the value of $J_{7,7\pm1}$, $J_{7,13}$ and Φ_R is also simultaneously correlated with the configuration of the endocyclic double bond between $C_{(4)}$ and $C_{(5)}$. Thus the observed values, $J_{6,7} = 1.8$ Hz, $J_{7,13} = 1.90$ Hz and $J_{7,13'} = 1.80$ Hz, for nobilin (I; in CDCl$_3$) indicate that nobilin belongs to germacra-1(10)-$trans$-

-4(5)-*cis*-dienolides (heliangolides)[6] (^{1}H-NMR spectrum of nobilin: Table I). The NOE-experiments carried out with the derivative of nobilin[1] *IX*, the spectrum (Table I) of which was suitable for this purpose also witnessed the mentioned configuration of the double bonds in nobilin. In a non-degased dilute CDCl$_3$ solution a 14% NOE was observed for the experiment H$_5$—{H$_{15}$}, and a 0% NOE for the experiment H$_1$—{H$_{14}$} (*cf.* also refs[5,8]). The acylation shifts of the signal of proton H$_6$ induced by the acylation of the hydroxyl at C$_{(3)}$ also support the structure *I* for nobilin (*cf.* Table II). In CDCl$_3$ solutions diamagnetic shifts $\Delta^{(3)}\delta$H$_6$ (R = Ac) = = +0·52 p.p.m. and $\Delta^{(3)}\delta$H$_6$(R = TAC) = +0·49 p.p.m. (TAC: CCl$_3$CONHCO—; TAI-method[9,10]) were observed; for the definition of the shifts see Table II. These shifts indicate the van der Waals effect of the hydroxyl group bound to C$_{(3)}$ on proton H$_6$ and indicate that the distance between the oxygen atom of the mentioned group and the proton H$_6$ is about 2 Å (ref.[11]). From an analysis of Dreiding models the realization of such conformational conditions for the van der Walls effect is possible only under the assumption of a *cis*-configuration of the double bond between C$_{(4)}$ and C$_{(5)}$. The spacial proximity of the oxygen atom bound at C$_{(3)}$ to H$_6$ is also confirmed by the chemical shift of H$_6$ (δH$_6$ = 4·22 p.p.m.) in 3-dehydronobilin (*IV*). The strong diamagnetic shift by 1·73 p.p.m. in relation to nobilin (*I*) (see Table I) can be explained by the elimination of the van der Waals effect and by the diamagnetic contribution of the anisotropic field of the carbonyl group (according to the value $J_{6,7}$ = 1·2 Hz the configuration of the double bond between C$_{(4)}$ and C$_{(5)}$ is *cis* also in 3-dehydronobilin (*IV*)). The absence of a significant paramagnetic shift δH$_5$ (*I*) − δH$_5$ (*IV*) also deserves mention, because it could be expected in analogy to the known shifts of the β-hydrogens of the double bonds in α,β-unsaturated ketones in cases when the carbonyl group and the double bonds are *trans* and coplanar

$$(\overset{(-)}{\text{CO—CH=CH—}} \leftrightarrow \overset{(+)}{\text{O—C=C—CH—}}).$$ The absence of this shift agrees well with the discussed effects because it leads to the assumption that the carbonyl group cannot be coplanar with the double bonds, which is in agreement with the conclusions obtained from the analysis of Dreiding models. The absence of conjugation is also indicated by the frequency of the carbonyl group in the IR spectrum of 3-dehydronobilin (1 709 cm^{-1}) as well as by its UV spectrum (λ_{max} 212 nm). The observed effects also confirm the originally proposed[2] β-configuration of the hydroxyl group on C$_{(3)}$. The sum of the mentioned facts shows that structure *I* is correct for nobilin.

During the chromatography of the crude nobilin fraction[1] on a silica gel column 3-dehydronobilin (*IV*) was eluted first, having m.p. 205°C, [α]$_D^{20}$ +136·6° and the composition C$_{20}$H$_{24}$O$_5$, also in agreement with the mass spectrum. As shown by the physical constants of this compound, by its IR and ^{1}H-NMR spectrum (Table I), as well as by the mixture melting points which was undepressed, this substance is identical with 3-dehydronobilin prepared[1] on oxidation of nobilin (*I*). In subsequent fractions nobilin (*I*) was eluted, closely accompanied by 3-epinobilin (*II*),

m.p. 137°C and the composition $C_{20}H_{26}O_5$. The IR spectrum of 3-epinobilin (*II*) contained absorption bands at 3260 cm^{-1} (free hydroxyl group), at 1764, 1405, 1153 and 888 cm^{-1} (α,β-conjugated exomethylene-γ-lactone), at 1704 and 1203 cm^{-1}

TABLE I
Characteristic Parameters of the ^{1}H-NMR Spectra of Substances *I—IV*

Substance[a]	H$_1$	H$_3$	H$_5$	H$_6$	H$_7$
I	5·26	4·46 $J_{3,2} = 3·8$ $J_{3,2'} = 2·8$	5·19 $J_{5,6} = 10·5$	5·95 $J_{6,5} = 10·5$ $J_{6,7} = 1·8$	2·94 $J_{7,8} = 10$
XVI[c]	5·32	5·22	5·15 $J_{5,6} = 10$	5·43 $J_{6,5} = 10$ $J_{6,7} = 1·6$	2·96 $J_{7,8} = 10$
IV[d]	5·44		5·17 $J_{5,6} = 10·3$	4·22 $J_{6,5} = 10·3$ $J_{6,7} = 1·2$	2·95 $J_{7,8} = 10·15$
IX[e]	5·28	4·42 $J_{3,2} = 3·3$ $J_{3,2'} = 3·3$	5·37 $J_{5,6} = 10$	6·00 $J_{5,6} = 10·1$ $J_{6,7} = 2·6$	2·56 $J_{7,8} = 9$ $J_{7,6} = 2·5$ $J_{7,11} = 12$
II[f]	5·24	4·58 $J_{3,2} = 5$ $J_{3,2'} = 11·5$	5·12 $J_{5,6} = 11$	4·79 $J_{5,6} = 11$ $J_{6,7} = 1·5-2$	2·92 $J_{7,8} = 10$
III[g]	2·82 $J_{1,2} = 4·5$ $J_{1,2'} = 10$	4·44 $J_{3,2} = 4·4$ $J_{3,2} = 2·5$	5·27 $J_{5,6} = 10·5$	6·37 $J_{6,5} = 10·4$ $J_{6,7} = 1·6$	2·87 $J_{7,8} = 10$

[a] Measured on a Varian HA-100 instrument; first-order analysis using double resonance experiments; chemical shifts in δ(TMS)-scale; splittings in Hz; solvent deuteriochloroform, internal standard tetramethylsilane (TMS); additional data given in the footnotes; [b] $J_{13,6} \neq 0$ (confirmed by double resonance); [c] H$_2$: 2·68 ($J_{2,3} \cong 2·6$; $J_{2,1} \cong 7$; $J_{2,2'} = 14$); H$_2'$: 2·12—2·38; H$_9$: 2·64 ($^2J \cong 13$); H$_{9'}$: 2·22—2·43; CH$_3$.COO—: 2·07; β-H (angelyl): 6·08 qq $|J_{2,3} + J_{2',3}| \cong \cong 5·2$; [d] 2 H$_2$: 3·19 d ($J = 8·5$); H$_1$: 5·44 bt ($J \cong 8·5$); H$_9$: 2·69 bdd ($J_{9,8} = 4·0$; $J_{9,9'} = 12$);

(ester group with α,β-conjugated double bond), and at 1650 cm^{-1} (double bond). In the mass spectrum the molecular peak at m/e 346 was not detected, but the fragment of m/e 246 (M − 100) was present, corresponding to the part with the split

TABLE I
(*Continued*)

H$_8$	H$_{13}$	H$_{13'}$	H$_{14}$	H$_{15}$
5·06	6·31^b	5·67	1·84	1·65
$J_{8,7} = 10·3$	$J_{13,7} = 1·9$	$J_{13',7} = 1·8$	$J_{14,1} = 1·2$	$J_{15,5} = 1·5$
$J_{8,9'} = 10·3$				
$J_{8,9} = 3·8$	$^2J = 0·9$	$^2J = 0·9$		
5·07	6·32^b	5·69	1·87	1·80
$J_{8,7} = 10·4$	$J_{13,7} = 1·95$	$J_{13',7} = 1·8$	$J_{14,1} = 1·1$	$J_{15,5} = 1·4$
$J_{8,9'} = 10·4$				
$J_{8,9} = 3·9$	$^2J = 0·9$	$^2J = 0·9$		
5·00	6·32^b	5·68	1·80	1·88
$J_{8,7} = J_{8,9'} = 10·5$	$J_{13,7} = 1·85$	$J_{13',7} = 1·7$		$J_{15,5} = 1·4$
$J_{8,9} = 4·05$	$^2J = 0·8$	$^2J = 0·8$		
3·74	4·38	3·83	1·81	1·73
$J_{8,7} = 9·2$	$J_{13,11} = 9·4$	$J_{13',11} = 6·8$	$J_{14,1} = 1·25$	$J_{15,5} = 1·4$
$J_{8,9} = 3·6$				
$J_{8,9'} = 10·9$	$^2J = 9·4$	$^2J = 9·2$		
5·10	6·28	5·66	1·88	1·79
	$J_{13,7} = 1·95$	$J_{13',7} = 1·75$		$J_{15,5} = 1·3$
	$^2J = 0·9$	$^2J = 0·9$		
5·08	6·31	5·67	1·58	1·80
$J_{8,7} = 10$	$J_{13,7} = 1·95$	$J_{13',7} = 1·6$		$J_{15,5} = 1·4$
$J_{8,9} = 3$	$^2J = 0·8$	$^2J = 0·8$		
$J_{8,9'} = 12$				

H$_{9'}$: 2·38 dd ($J_{9',8} = 10·65$; $J_{9,9'} = 12$); β-H (angelyl): 6·07 qq; ^{e} H$_2$: 2·60 ddd ($J_{2,3} = 3·5$; $J_{2,1} = 10$; $J_{2,2'} = 14·5$); H$_{2'}$: 2·21 ddd ($J_{2',3} = 3·5$; $J_{2',1} = 7·5$; $J_{2',2} = 14·5$); H$_9$: ~2·56; H$_{9'}$: 2·12 t ($J_{9',8} = J_{9',9} = 11·3$); H$_{11}$: 3·45 ddd ($J_{11,7} = 11·7$; $J_{11,13} = 9·8$; $J_{11,13'} = 7·1$); ^{f} H$_9$: 2·30 t ($J_{9,8} = J_{9,9'} \cong 11·5$); β-H (angelyl): 6·07 qq; ^{g} H$_2$: 2·44 dt ($J_{2,1} \cong J_{2,3} \cong 4·5$; $J_{2,2'} = 15$); H$_{2'}$: 1·69; H$_9$: 2·59 dd ($J_{9,8} = 3$; $J_{9,9'} = 13$); H$_{9'}$: 1·38 dd ($J_{9',8} = 12$; $J_{9',9} = 13$); β-H (angelyl): 6·08 qq.

off C_4H_7COOH, as well as the fragments m/e 83 ($C_4H_7CO^+$) and 55 ($C_4H_7^+$). The most polar substance isolated was 1,10-epoxynobilin (*III*), m.p. 192°C, $[\alpha]_D^{20} \pm 0^0$ and the composition $C_{20}H_{26}O_6$. The IR spectrum of 1,10-epoxynobilin (*III*) contained absorption bands at 1763, 1407 and 1154 cm^{-1} (α,β-unsaturated exomethylene-γ-lactone), 3605 and 3480 cm^{-1} (free hydroxyl group), at 1714 cm^{-1} (ester group conjugated with a double bond) and at 1648 and 1663 cm^{-1} (double bond). The mass spectrum contained the molecular peak at m/e 362 and fragments at m/e 344 (M − 18), 279 (M − 83), 262 (M − 100), 83 ($C_4H_7CO^+$) and 55 ($C_4H_7^+$).

Characteristic parameters of the ^1H-NMR spectra of 3-epinobilin (*II*) and 1,10-epoxynobilin (*III*) are given in Table I. The assignment of the spectra was carried out by frequency swept decoupling experiments. The structures of both substances and their relatedness with the structure of nobilin (*I*) followed directly from the observed distribution of the chemical shifts and the topological continuity of the vicinal and long-range couplings and from their comparison with the spectra of nobilin (*I*) and some of its derivatives (Table I). Characteristic are again the coupling constants $J_{7,13} = 1.95$ (transoid), $J_{7,13'} = 1.75$ (cisoid) and $J_{6,7} \cong 1.5-2$ Hz for 3-epinobilin, and $J_{7,13} = 1.95$ (transoid), $J_{7,13'} = 1.6$ (cisoid) and $J_{6,7} = 1.6$ Hz for 1,10-epoxynobilin (*III*) indicating − with respect to the above mentioned rule[7] − the typical stereochemistry of heliangolides with a $C_{(6)}$-*trans* lactone and a *cis* double bond between $C_{(4)}$ and $C_{(5)}$. The position of the hydroxyl group was in both case- also confirmed by acylation shifts induced by *in situ* acylation with trichloroacetyls isocyanate in $CDCl_3$ solutions. These shifts are summarized in Table II.

Table II

Acylation Shifts of Signals of Characteristic Protons of Table I Induced by *in situ* Acylation of $C_{(3)}$—OH Groups with Trichloroacetyl Isocyanate (TAI)[a]

| Substance	H_1	H_3	H_5	H_6	H_7	H_8	H_{13}	$H_{13'}$	H_{14}	H_{15}
I	—	−0·90	−0·04	+0·49	−0·03	−0·01	−0·02	−0·03	−0·03	−0·26
XXVII	−0·06	−0·76	+0·04	+0·48	−0·02	−0·01	−0·02	−0·02	−0·03	−0·15
IX	—	−0·98	−0·08	+0·28	—	—	0·00	−0·03	+0·01	−0·15
II	0·00	−1·05	−0·12	+0·05	0·00	0·00	−0·03	−0·01	+0·01	−0·20
III	−0·08	−0·99	−0·07	+0·63	−0·05	−0·07	0·00	−0·04	−0·07	−0·14

[a] Acylation shifts are defined as $\Delta^{(i)}\delta H_{(j)} = \delta H_{(j)}(C^{(i)}—OH) - \delta H_{(j)}(C^{(i)}—OR)$ where (*i*) denotes the position of the OH group being acylated, (*j*) denotes the proton the signal of which is shifted by acylation in the position (*i*) (in the arbitrary numbering) and R denotes the acyl group (in this case $i = 3$ and R = $CO.NH.CO.CCl_3$).

The relatively small γ-acylation shifts (RO—C(α)—C(β)—C(γ)...) of the vinyl proton (similar effects for the vinyl protons in vinylic alcohols are mentioned in refs[9,12]) and relatively larger γ-shifts of methyl protons H_{15} (probably due to various anisotropic effects of the TAC group) are characteristic of the position of the hydroxyl group in $C_{(3)}$. The relatively high shift of proton H_6 and the indistinct acylation shift $\Delta^{(3)}\delta H_6$ (TAC) in the case of 3-epinobilin (*II*) is in agreement with the 3α-configuration of its hydroxyl group, because it corresponds to the absence of the already mentioned strong van der Waals effect, characteristic of the 3β-configuration in nobilin (*I*). In 1,10-epoxynobilin (*III*) a $\Delta^{(3)}\delta H_6$ (TAC) = +0·63 is again present, indicating a 3β-configuration of the hydroxyl group.

In 1,10-epoxynobilin (*III*) a considerable chemical shift of proton $H_6(\delta H_6 = 6\cdot37$ p.p.m.) is observed, highest in the series of nobilin derivatives. The paramagnetic contribution causing its shift stems evidently from the anisotropy of the three-membered oxiran ring. This distinct shift has also been observed in other heliangolides, as for example in 1,10-epoxyprovincialin (*X*) ($\delta H_6 = 6\cdot18$ p.p.m.)[5] or erioflorin acetate (*XI*) ($\delta H_6 = 6\cdot11$ p.p.m.)[12]. This shift can be rationalized assuming such a conformation in which the endocyclic double bonds in nobilin (*I*) have a conformation with a transannular cross-conjugation if passing to 1,10-epoxynobilin (*III*) under preservation of the conformation of C-homocyclic σ-bonds with an *exo*-configuration of the epoxide oxygen. In nobilin (*I*) proton H_6 is located in the diamagnetic part of the shielding field of the double bond $\Delta^{1(10)}$ (above the plane of the bond). This possible effect can be eliminated by epoxidation and substituted by the paramagnetic effect (in the plane of the three membered ring, by the ring-current effect). This stereochemistry of 1,10-epoxyheliangolides has also been found in a number of cases, as for example in heliangin[13] (*XII*), in the 1,10-epoxyprovincialin[5] (*X*) already mentioned, and in erioflorin acetate[12] (*XI*). The observed splitting in the multiplets H_1: $J_1 = 4\cdot5$ and $J_2 = 10$ Hz, which also corresponds well to 3β-hydroxy- -1,10-epoxy-heliangolides of the erioflorin acetate (*XI*) type (H_1:2·62 dd, $J_1 = 4$, $J_2 = 10$) (ref.[12]) are also in agreement with this configuration. Therefore we suppose that the configuration of the fragment $C_{(1)}$—$C_{(10)}$ is the same in 1,10-epoxynobilin (*III*) as in erioflorin[5,12] (*XIII*), heliangin[6,13] (*XII*) and related heliangolides. The sum of all the facts mentioned shows that formulas *II*, *III* and *IV* belong to the newly described substances, *i.e.* 3-epinobilin, 1,10-epoxynobilin and 3-dehydronobilin, respectively.

The absolute configuration of nobilin and its analogues has not as yet been determined unambiguously. Although the relative stereochemistry of the lactone ring is known the Cotton effects of the lactone chromophore in heliangolides do not enable an unambiguous decision about the absolute configuration on the basis of empirical correlations, as for example Geissman's rule[14,15], as already indicated by Herz and Wahlberg[5]. Certain information on absolute configuration of the mole-
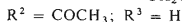
cule could afford the Cotton effect of the *trans*-annular conjugated double bonds. In germacra-1(10)-*trans*-4(5)-trans-dien-6,12-olides ($C_{(6)}$-germacrolides[6]) with 7αH configuration (in the meaning of the absolute configuration shown in the formulae *I—IV* for instance) the Cotton effect of this chromophore ranges from 220 to 227 nm, with average $\Delta\varepsilon$ +25 to +30. These cases are conformationally uniform and possess the so-called transannular-cross-conjugation of the endocyclic double bonds. The Cotton effect of nobilin and its analogues in the 202—210 nm region, similarly as eucannabinolide[4] (*VI*) have $\Delta\varepsilon$ −16·2 to −25·6. From the ¹H-NMR spectra and the Dreiding models it follows that these substances should possess an optimum conformation of their ten-membered ring, in which the endocyclic double bonds also assume a transannular-cross-conjugation, but with the opposite arrangement than in $C_{(6)}$-7αH-germacrolides[6], when assuming the same absolute configuration of the
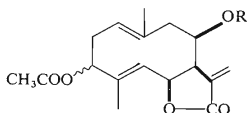
VI, R = COC(CH₂OH)=CHCH₂OH
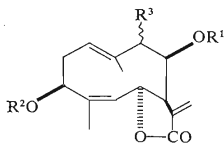
VII, R¹ = COC=CHCH₂OH
      |
     CH₂OCOC=CHCH₃
            |
           CH₂OH
    R² = COCH₃; R³ = H
VIII, R¹ = COC(CH₂OH)=CHCH₂OH
     R² = COCH₃; R³ = H
XIV, R¹ = COC(CH₃)=CHCH₃
     R² = COCH₃; R³ = OH
XV, R¹ = H; R² = COCH₃
     R³ = OCOC   CHCH₃
         H₃C O
XVII, R¹ = COCH(CH₃)₂
      R² = COCH₃; R³ = H
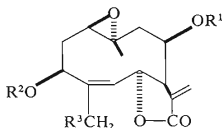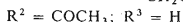
X, R¹ = COC=CHCH₂OH
         |
     CH₂OCOC=CHCH₃
             |
           CH₂OH
    R² = COCH₃; R³ = H
XI, R¹ = COC(CH₃)=CH₂
     R² = COCH₃; R³ = H
XII, R¹ = COC(CH₃)=CHCH₃; R², R³ = H
XIII, R¹ = COC(CH₃)=CH₂; R², R³ = H
XVIII, R¹, R² = COC(CH₃)=CH₂; R³ = H
XIX, R¹ = COC(CH₃)=CH₂
     R² = COCH₃; R³ = OH
XX, R¹ = COC(CH₃)=CH₂; R² = H; R³ = OH
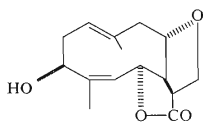
IX
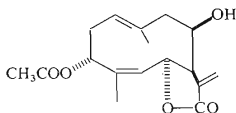
XVI
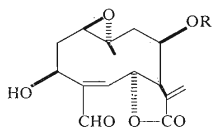
XXI, R = COC(CH₃)=CH₂
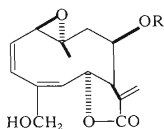
XXII, R = COC(CH₂OH)=CHCH₃
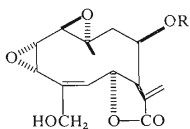
XXIII, R = COC(CH₃)=CHCH₃
XXIV, R = COC=CHCH₃
        CH₂OCOCH₃
XXV, R = COC=CHCH₂
        CH₃ OCOCH₃
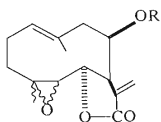
XXVI, R = COC(CH₂OH)=CHCH₂OH
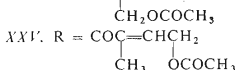
substituents on $C_{(7)}$ (7αH). Hence, the sign of the Cotton effect belonging to the transannular conjugation of the double bonds (while the configuration of the centre $C_{(7)}$ would be the same) would respect the sign of the cross-conjugation of the endocyclic double bonds. Under the assumption that the conformation of the $C_{(6)}$-γ-lactone ring and the endocyclic double bonds in these substances are correlated in this sense, the Cotton effect due to the crossed transannular conjugation could indicate membership of the series 7αH or 7βH. In the case of nobilin and its analogues this would permit the proposal of the absolute configuration represented by formulas *I* to *IV*.

In view of their structure all the substances investigated — nobilin (*I*), 3-epinobilin (*II*), 1,10-epoxynobilin (*III*) and 3-dehydronobilin (*IV*) — belong among germacra--1(10)-*trans*-4(5)-*cis*-dienolides (heliangolides)[6]. Up to now altogether seventeen heliangolides have been described, of which six are substances — provincialin[5] (*VII*), eucannabinolide[4,5] (*VIII*), eupatocunin[16,17] (*XIV*), eupatocunoxin[17] (*XV*), eupaformonin[18] (*XVI*) and peucephyllin[19] (*XVII*) — with both endocyclic double bonds "free", and ten compounds (heliangin[13] (*XII*), erioflorin[12,5] (*XIII*), erioflorin

acetate[12] (*XI*), erioflorin methacrylate[12] (*XVIII*), eriophyllin[20] (*XIX*), eriophyllin B (*XX*) (ref.[20]), eriophyllin C (*XXI*) (ref.[20]), punctaliatrin[21] (*XXII*), liscundin[2] (*XXIII*), liscunditrin[22] (*XXIV*) and eleganin[22] (*XXV*) which instead of a *trans* double bond between $C_{(1)}$ and $C_{(10)}$ contain a *trans* oxide group among the mentioned carbon atoms and a *cis* double bond between $C_{(4)}$ and $C_{(5)}$, and, so far a single substance — liatripunctin[22] (*XXVI*) — with a *trans* double bond between $C_{(1)}$ and $C_{(10)}$ and with a *cis* epoxide at $C_{(4)}$ and $C_{(5)}$. All the heliangolides described so far have their γ-lactone group conjugated with the exomethylene double bond and have it closed in all these native substances at $C_{(6)}$. In the majority of heliangolides an oxygen function is located at $C_{(3)}$, predominantly β-oriented. All heliangolides have so far contained a β-oriented hydroxyl group on $C_{(8)}$ (free or esterified), but nobilin (*I*) and substances *II* to *IV* have an α-oriented $C_{(8)}$—O bond and thus represent the sole representatives with such a substitution in the heliangolide group.

Nobilin (*I*), 1,10-epoxynobilin (*III*) and 3-dehydronobilin (*IV*) possess a considerable *in vitro* inhibitory activity against human tumor cells of *carcinoma cervicis uteri* (HeLa) and *nasopharynx carcinoma*[23] (KB).

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. For column chromatography silica gel according to Pitra and Štěrba[24] (30—60 μm, deactivated by addition of 11% of water) was used. For TLC silica gel G (Merck) was used. The IR spectra were measured in chloroform on a Unicam SP-200 and Zeiss UR-10 (Jena) spectrophotometer. The ^{1}H-NMR spectra were measured on a Varian HA-100 apparatus. The mass spectra were measured on an AEI MS 902 instrument. Optical rotation was determined with an objective polarimeter in methanol. Circular dichroism was measured on a Roussel-Jouan Dichrographe CD 185 in methanol.

Separation of the Lactone Fraction from the Flowers of *Anthemis nobilis* L.

Crude nobilin fraction (3·2 g), prepared in the described manner[1], was chromatographed on a silica gel column (250 g), as shown in Table III.

3-Dehydronobilin (IV) was obtained by crystallization of fraction 2 (Table III), m.p. 205°C (ethanol, chloroform), $[\alpha]_D^{20} +136·6°$ (*c* 0·526). IR spectrum (cm^{-1}): 1768, 1407, 1148 (exomethylene-γ-lactone), 1662, 1650 (double bond), 1709 (ketone and α,β-unsaturated ester). Mass spectrum: 344 (M$^+$), 244 (M — 100), 83 ($C_4H_7CO^+$), 55 ($C_4H_7^+$). CD spectrum: 202 nm, $\Delta\varepsilon$ —23·9; 223 nm, $\Delta\varepsilon \pm 0$; 301 nm, $\Delta\varepsilon$ +5·5; 335 nm, $\Delta\varepsilon \pm 0$. The mixture melting point with a sample of 3-dehydronobilin (*IV*) melting at 202—203°C and prepared on oxidation of nobilin[1] (*I*) was undepressed.

Nobilin (I) was obtained by crystallization of fraction 3 (Table III); m.p. 178°C (ethanol), undepressed on admixture of a reference sample of nobilin (*I*). The IR spectrum and ^{1}H-NMR spectrum of this sample were identical with analogous spectra of nobilin (*I*). CD spectrum: 209 nm, $\Delta\varepsilon$ —26·3; 223 nm, $\Delta\varepsilon \pm 0$; 233 nm, $\Delta\varepsilon$ +4·6; 300 nm, $\Delta\varepsilon \pm 0$.

3-Epinobilin (II) was obtained by repeated chromatography of fraction 4 (Table III); m.p. 137°C. IR spectrum (cm^{-1}): 3260 (hydroxyl), 1764, 1405, 1153, 888 (exomethylene-γ-lactone),

TABLE III

Chromatography of the Lactone Fraction

Fraction	Solvent	Volume, l	Residue, mg	Substance
1	benzene	1·5	42	—
2	benzene–ether 19 : 1	0·5	51	*IV*
3	benzene–ether 9 : 1	2·3	2 112	*I*
4	benzene–ether 9 : 1	0·05	62	*II*
5	benzene–ether 9 : 1	0·15	83	—
6	benzene–ether 9 : 1	0·15	286	*III*
7	benzene–ether 9 : 1	0·20	222	—
8	benzene–ether 1 : 1	0·5	178	—

1704, 1203 (α,β-unsaturated ester), 1650 (double bond). Mass spectrum: 246 (M $-$ 100), 83 ($C_4H_7CO^+$), 55 ($C_4H_7^+$). CD spectrum: 205 nm, $\Delta\varepsilon$ $-16\cdot2$; 220 nm, $\Delta\varepsilon \pm0$; 230 nm, $\Delta\varepsilon$ $+2\cdot5$; 295 nm, $\Delta\varepsilon \pm0$. For $C_{20}H_{26}O_5$ (346·4) calculated: 69·34% C, 7·57% H, 0·29% H act.; found: 69·59% C, 7·38% H, 0·25% H act.

1,10-*Epoxynobilin* (*III*) was obtained by crystallization of fraction 6 (Table III); m.p. 192°C, $[\alpha]_D^{20} \pm0°$. IR spectrum (cm^{-1}): 3605, 3480 (hydroxyl), 1763, 1707, 1154 (exomethylene-γ-lactone), 1648, 1663 (double bond), 1714 (α,β-unsaturated ester). Mass spectrum: 362 (M$^+$), 344 (M $-$ 18), 262 (M $-$ 100), 244 (M $-$ 100$-$18), 83 ($C_4H_7CO^+$), 55 ($C_4H_7^+$). CD spectrum: 237 nm, $\Delta\varepsilon +1\cdot6$; 290 nm, $\Delta\varepsilon \pm0$. For $C_{20}H_{26}O_6$ (362·4) calculated: 66·28% C, 7·23% H, 0·36% H act.; found: 66·47% C, 7·53% H, 0·28% H act.

Eucannabinolide (*VIII*)

CD spectrum: 210 nm, $\Delta\varepsilon -18\cdot0$; 245 nm, $\Delta\varepsilon \pm0$; 260 nm, $\Delta\varepsilon +0\cdot45$.

REFERENCES

1. Benešová V., Herout V., Šorm F.: This Journal **29**, 3096 (1964).
2. Benešová V., Samek Z., Herout V., Šorm F.: Tetrahedron Lett. *1970*, 5017.
3. Samek Z.: Tetrahedron Lett. *1970*, 671.
4. Drożdż B., Grabarczyk H., Samek Z., Holub M., Herout V., Šorm F.: This Journal **37**, 1546 (1972).

5. Herz W., Wahlberg I.: J. Org. Chem. *38*, 2485 (1973).

6. Rogers D., Moss G. P., Neidle S.: Chem. Commun. *1972*, 142.

7. Samek Z.: Unpublished results.

8. Tori K., Horibe I., Yoshioka H., Mabry T. J.: J. Chem. Soc. *1971*, 1084.

9. Goodlett V. W.: Anal. Chem. *37*, 431 (1965).

10. Trehan I. R., Monder C., Bosse A. K.: Tetrahedron Lett. *1968*, 67.

11. Yoshioka H., Mabry T. J., Irwin M. A., Geissman T. A., Samek Z.: Tetrahedron *27*, 3317 (1971).

12. Gnecco S., Poyser J. P., Silva M., Sammes P. G., Tyler T. W.: Phytochemistry *12*, 2469 (1973).

13. Nishikawa M., Kamiya K., Takabatake A., Oshio H., Tomiiey Y., Nitta I.: Tetrahedron *22*, 3601 (1966).

14. Waddell T. G., Stöcklin W., Geissman T. A.: Tetrahedron Lett. *1969*, 1313.

15. Stöcklin W., Waddell T. G., Geissman T. A.: Tetrahedron *26*, 2397 (1970).

16. Kupchan S. M., Maruyama M., Hemingway R. J., Hemingway J. C., Shibuya S., Fujita T., Cradwick P. D., Hardy A. D. U., Sim G. A.: J. Amer. Chem. Soc. *93*, 4914 (1971).

17. Kupchan S. M., Maruyama M., Hemingway R. J., Hemingway J. C., Shibuya S., Fujita T.: J. Org. Chem. *38*, 2189 (1973).

18. McPhail A. T., Onan K. D., Lee K.-H., Ibuka T., Huang H.-Ch.: Tetrahedron Lett. *1974*, 3203.

19. Begley M. J., Pattenden G., Mabry T. J., Miyakado M., Yoshioka H.: Tetrahedron Lett. *1975*, 1105.

20. Torrance S. J., Geissman T. A., Chedekel M. R.: Phytochemistry *8*, 2381 (1969).

21. Herz W., Wahlberg I.: Phytochemistry *12*, 1421 (1973).

22. Herz W., Sharma R. P.: Phytochemistry *14*, 1561 (1975).

23. Hładoń B.: Personal communication.

24. Pitra J., Štěrba J.: Chem. Listy *56*, 544 (1962).

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