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On Centaur Z, a Compound of Polyenynic Type in *Centaurea montana* L.

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Submitted in honour of the ninetieth birthday of Professor *Hans von Euler-Chelpin*

A highly unsaturated and very unstable compound, called Centaur Z, has been isolated from the roots of *Centaurea montana* L. The empirical formula was found to be $C_{21}H_{26}O_4$. It was further demonstrated that Centaur Z is a disubstituted ene-diyne-diene

$$R_1-CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH-R_2$$

where neither R_1 nor R_2 contain any $C=C$ or $C\equiv C$ bonds. As to the nature of R_1 and R_2 it was also established that the *sum* of R_1 and R_2 is equal to the sum of C_7H_{14} and two acetoxy groups. Centaur Z is thus a diacetate of a diol $C_{17}H_{20}(OH)_2$ with a chromophoric system as given above. Further it was shown that Centaur Z has an all-*trans* structure.

By investigating the roots of *Centaurea montana* L. a new compound, here called Centaur Z ("CZ"), of polyenynic type was discovered. The isolation of this compound and the partial determination of its structure will be described below. At first, however, a brief review of the occurrence of highly unsaturated compounds in *Centaurea cyanus* L. and related plants will be given.

The Centaur X and Y compounds. In 1949 Löfgren [1] reported the existence of two groups of compounds, the so-called Centaur X and Y groups, with characteristic but different U.V.-spectra, in the extract from stalks and leaves of *Centaurea cyanus* L. Further investigations [2] indicated that the compounds in the Centaur Y group are probably of polyenic type and at least two in number, denoted by CY_1 and CY_2. The compounds have identical U.V.-spectra but differ as to their partition coefficients *. It was also established that at least four compounds, called CX_1, CX_2, CX_3, and CX_4 belong to the Centaur X group. CX_1 and CX_2 form one subgroup and CX_3 and CX_4 another with regard to the partition coefficient (distribution as above), while from their

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* Distribution between cyclohexane and 90 % methanol.

U.V.-spectra, CX_1 is identical with CX_3 and CX_2 with CX_4. Attempts were made to isolate the above mentioned compounds in a pure form from the green parts of *C. cyanus* L. The difficulties arising from the extremely small quantities in which the compounds are present, and the great resemblance of their physico-chemical properties made, however, the isolation unsuccessful.

In 1957 Bohlmann *et al.*[3] isolated a hydrocarbon, $C_{17}H_{18}$, from the roots of *Artemisia vulgaris* L. This hydrocarbon they showed to be identical with the compound CX_3 from *C. cyanus* L. By analysis [3] the formula for CX_3 was found to be 1,7,9-heptadecatriene-11,13,15-triyne and this formula was later confirmed by synthesis [4].

In *C. macrocephala* Puschk. two U.V.-identical compounds, CX_1 and CX_1', were isolated by Bohlmann *et al.*[5] in 1958. The compound CX_1 has not been isolated in a pure form, but the authors consider it to have a chromophore of triyne-diene type, and the I.R.-spectrum indicates the presence of at least one ester group. CX_1' was isolated in a pure state and was ascribed the formula 1,3-diacetoxy-5,7-pentadecadiene-9,11,13-triyne. This structure was based on results from ultraviolet and elementary analyses.

In the same plant Bohlmann *et al.*[5] found CX_2 and another compound CX_2', having identical U.V.-spectra. CX_2 was isolated in a pure form and found by analyses (spectra, hydrogenation, degradations) to be 1,3-diacetoxy-5,7,13-pentadecatriene-9,11-diyne. Final proof of this structure was given by Ruhnke [6] by synthesis. Concerning the compound CX_2' Bohlmann *et al.*[5] did not succeed in obtaining it in a quite pure form, but from spectra and a crystalline ester as obtained by reaction with *p*-phenylazobenzoyl chloride, CX_2' was thought to be a compound containing a hydroxy group, an ester group, and a chromophore of ene-diyne-diene type.

Bohlmann *et al.*[5] also tried to isolate CX_4 from *C. cyanus* L. but like Hellström and Löfgren [2] (*cf.* above) they did not succeed. However, by working with *C. ruthenica* Lam., Bohlmann *et al.*[5] were able to obtain the compound in the pure state. Furthermore Bohlmann and co-workers [5] found by analyses CX_4 to be 1,7,9,15-heptadecatetraene-11,13-diyne and later Bohlmann and Herbst [7] confirmed the structure by synthesizing CX_4. By this synthesis CX_4 was also shown to have an all-*trans* configuration.

Isolation of CZ. As stated above a new compound, Centaur Z (CZ), of polyenynic type was found in the roots of *Centaurea montana* L. Thus the extracts gave a characteristic U.V.-spectrum indicating the compound to be of ene-diyne-diene type (*cf.* below) and further investigations showed that the compound has not earlier been described.

CZ was extracted from the roots of the plant by a procedure that diverged from the commonly used methods, the fresh roots being treated with chloroform in the presence of anhydrous sodium sulphate to bind the water-contents of the material (formation of hydrated sodium sulphate). By filtering the mixture, only one liquid phase — the chloroform solution — was obtained, the water being bound to the sodium sulphate.* After filtering, the solids

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* This type of extraction, in which anhydrous sodium sulphate is used to bind the water, is considered by us to be generally applicable in extracting lipophilic compounds present in water-containing materials from the vegetable or animal kingdom. Instead of chloroform other lipophilic solvents, non-miscible with water, may naturally be used.

— consisting of vegetable material and hydrated sodium sulphate — was re-extracted with chloroform. The combined chloroform extracts were freed from the solvent by distillation in *vacuo*, and the residue dissolved in a mixture of carbon tetrachloride and cyclohexane (*cf.* experimental part). This solution, when chromatographed on aluminium oxide, gave a fast-moving, pale yellow, nonpolar fraction consisting of Sörensen's[8] 1,11-tridecadiene-3,5,7,9-tetrayne, earlier found in *Coreopsis* species[5,8,9]. A relatively slow-moving fraction, containing a compound with a very characteristic U.V.-spectrum, appeared then in the elution fluid. This fraction was however contaminated with the diene-tetrayne (see above) and therefore the chromatographic procedure had to be repeated twice. In this way a colourless fraction, free from the diene-tetrayne, was obtained, giving the characteristic spectrum of CZ (*cf.* Fig. 1). This fraction was evaporated under reduced pressure. The residue was crystallized from pentane, giving colourless needles of m.p. 33°C. Further recrystallizations did not alter the product as to its melting point or elementary composition (*cf.* below). A yield of 20 mg substance was obtained from 9 kg of fresh roots.

The compound is extremely sensitive to atmospheric oxygen and light. So, for instance, the compound will soon change into a brown, amorphous mass — insoluble in pentane and ether — when exposed to the air at room temperature. (This change is rapidly noticeable already in the dark.) *

Analysis of the U.V.-spectrum of CZ. The U.V.-spectrum of CZ is shown in Fig. 1. The wavelength of the maxima ($\lambda_{\max}$), their extinctions ($\varepsilon_{\max}$), their frequencies ($\nu_{\max}$) and the differences between adjacent maxima ($\Delta\nu_{\max}$) are found in Table 1. The relation between the positions of the maxima and the number of conjugated double bonds (or triple bonds) in unbranched chro-

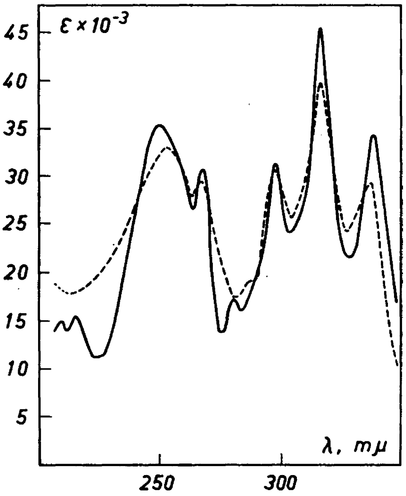

Fig. 1. Ultraviolet absorption of:
———— Centaur Z in cyclohexane
-------- Oenanthotoxin in alcohol[16]

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* Due to the difficulty in excluding atmospheric oxygen completely while handling the compound, about 9 of the 20 mg (see above) were later lost. Thus, all operations in this work were performed with approximately 11 mg of the *pure* compound.

Table 1. Data for Centaur Z from U.V.-measurements in cyclohexane.

ε_{max}	34 000	44 900	30 800	16 700	30 500	35 100	15 200	14 700
λ_{max}, mμ	338.5	316.5	297.5	280.4	267.5	249.5	215.0	208.6
$\nu_{max} \times 10^{-12}$, sec^{-1}	885.7	947.2	1008	1069	1121	1202	1394	1437
$\Delta\nu \times 10^{-12}$, sec^{-1}	62 61 62 52 81 193 43							

mophoric chains was studied by Hausser *et al.*[10], Dewar,[11] Dale,[12,13] Kuhn[14] and Hirayama[15]. Only one of these authors, *viz.* Dale,[13] discussed mixed chromophores composed of both double and triple bonds, the so-called enynic chromophore type. The position of the peaks as well as the $\Delta\nu_{max}$-values of the CZ-spectrum (*cf.* Fig. 1 and Table 1) show (Dale[13]) that the CZ-chromophore with the greatest probability is an unbranched ene-diyne-diene:

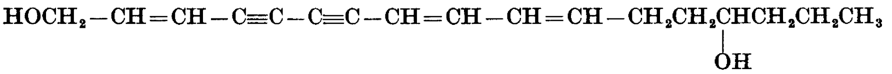
$$-CH{=}CH-C{\equiv}C-C{\equiv}C-CH{=}CH-CH{=}CH-$$

For such a chromophore, Dale states that the first peak in the main band (λ_1-band) is to be found near 336 mμ, and the first peak in the first over-tone (λ_2-band) near 266 mμ. The position of the latter peak is so highly characteristic for the number of conjugations and also for the sequence of the ethylenic and acetylenic bonds involved, that already this peak itself will decide the feature of the chromophore. Corresponding values for the CZ-spectrum are 338 mμ and 267 mμ. Other chromophores than that proposed here, would have the first peak in the λ_2-band situated far away from 266 mμ (*cf.* Dale[13]). Furthermore, the proposed chromophore should, contrary to many others of related type, show *two* acetylenic spacings each equal to 61—62 $\times$ 10^{12} sec^{-1} in the main band. The CZ-values are 62 $\times$ 10^{12} sec^{-1} and 61 $\times$ 10^{12} sec^{-1}.

Concerning the second over-tone (λ_3-band) no *vibrational* structure has hitherto been observed for compounds containing the proposed chromophoric system. The reason is probably that the investigators have worked in polar solvents (*e.g.* ethanol) or their spectrometers have not been able to record the vibrational structure in this short-wave region of the ultraviolet. For CZ in cyclohexane (see Fig. 1) two vibrational peaks in the λ_3-band was observed. It is interesting to note that the corresponding $\Delta\nu_{max}$-value is *characteristically ethylenic, i.e.* $\Delta\nu_{max}$ is equal to 43 $\times$ 10^{12} sec^{-1} (*cf.* Table 1).

As a further proof of the correctness of the proposed structure of the CZ-chromophore, seven compounds with this type of chromophore (see below) were compared with respect to their U.V.-spectra. It was found that these spectra are almost indistinguishable from each other and from the CZ-spectrum. The compounds compared are:

a. *Oenanthotoxin* (from *Oenanthe crocata* L.)



$$\mathrm{HOCH_2{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2CH_2\underset{\displaystyle |\atop \displaystyle OH}{C}HCH_2CH_2CH_3}$$

(Spectrum and constitution: see Anet *et al.*[16] and Bohlmann and Viehe [17]; *cf.* Fig. 1.)

b. *Aethusin* (from *Aethusa cynapium* L.)

$$\mathrm{CH_3{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2CH_3}$$

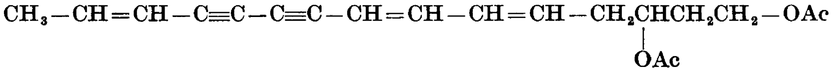
(Spectrum and constitution: see Bohlmann *et al.*[18])

c. *Oenanthetol* (from *Oenanthe crocata* L.)

$$\mathrm{HOCH_2{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2CH_2CH_2CH_2CH_2CH_3}$$

(Spectrum and constitution: see Anet *et al.*[16] and Hill *et al.*[19])

d. *Centaur* X_2 (from *Centaurea macrocephala* Puschk.)



$$\mathrm{CH_3{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2\underset{\displaystyle |\atop \displaystyle OAc}{C}HCH_2CH_2{-}OAc}$$

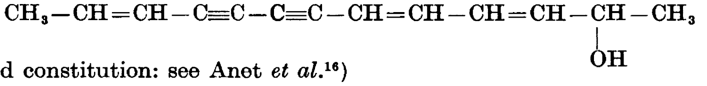
(Spectrum and constitution: see Bohlmann *et al.*[5] and Ruhnke [6].)

e. *Centaur* X_4 (from *Centaurea ruthenica* L.)

$$\mathrm{CH_3CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2CH_2CH_2CH_2{-}CH{=}CH_2}$$

(Spectrum and constitution: see Bohlmann *et al.*[5])

f. *3,5,11-tridecatriene-7,9-diyne-2-ol* (synthetic)



$$\mathrm{CH_3{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}\underset{\displaystyle |\atop \displaystyle OH}{C}H{-}CH_3}$$

(Spectrum and constitution: see Anet *et al.*[16])

g. *Aethusanol B* (from *Aethusa cynapium* L.)

$$\mathrm{HOCH_2{-}CH{=}CH{-}C{\equiv}C{-}C{\equiv}C{-}CH{=}CH{-}CH{=}CH{-}CH_2CH_3}$$

(Spectrum and constitution: see Bohlmann *et al.*[18])

Analysis of the I.R.-spectrum of CZ. Two bands, one at 2119 cm^{-1} and the other at 2183 cm^{-1} (see Fig. 2) are characteristic of the group

$$-C{\equiv}C-C{\equiv}C-$$

Thus Aethusin, Aethusanol B, CX_2 and CX_4*, each of which has this

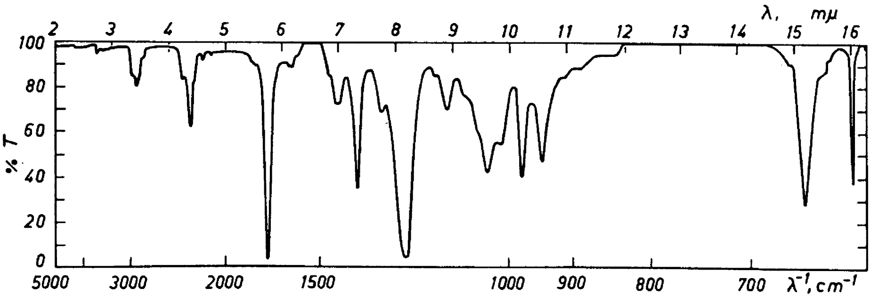


Fig. 2. Infrared spectrum of Centaur Z in carbon tetrachloride.

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* For Oenanthotoxin, Oenanthetol and 3,5,11-tridecatriene-7,9-diyne-2-ol, all of which have the same chromophoric system (equal to Aethusin, Aethusanol B, CX_2, and CX_4), no adequate I.R.-spectra have been published.

structural detail as a part of its total chromophoric system (*cf.* above), show the same two bands (Aethusin [18]: 2120 and 2200 cm^{-1}, Aethusanol B [18]: 2120 and 2200 cm^{-1}, CX_2[5,6]: 2125 and 2190 cm^{-1}, CX_4[5,7]: 2115 and 2190 cm^{-1}).

From chemical analysis (see below) it was shown that CZ contains acetoxy or propionyloxy groups. From the I.R.-spectrum it is evident that *CZ has at least one acetoxy group but no other type of acyloxy group*. The bands *significant* for the presence of the acetoxy group are found at 1364, 657, and 624 cm^{-1}. The 1364 cm^{-1} band is due to C—H deformation of the methyl in the acetoxy group [20,21]. Of the two other bands, the one at 624 cm^{-1} is considered to be connected with the acetyl part of the acetate-skeleton [22] whilst that at 657 cm^{-1} might be due to the entire framework CH_3COO [22]. The very strong band at 1742 cm^{-1} arises from carbonyl stretching vibration. In acetates the stretching vibration frequency of C=O lies at 1740 cm^{-1} (*cf.* Thompson and Torkington [22]), higher than in alkyl ketones or formates. Furthermore CZ has two bands, one at 1016 cm^{-1} and another at 1041 cm^{-1}, which are very probably associated with the acetoxy group [21-23]. For CX_2, which is a *diacetate*, these two bands are found at 1024 and 1047 cm^{-1}; the I.R.-spectra of CZ and CX_2 in the region 900—1100 cm^{-1} are almost identical.

Since the six acetate bands are strikingly strong in CZ it could be expected that the band associated with the ether-oxygen in the acetoxy group would show a pronounced high intensity, too. This is also the case, and the band referred to is found at 1222 cm^{-1}. Moreover, the position of the band is highly characteristic for the acetates. [22]

Owing to the high intensity of the acetate bands it seemed very probable that CZ contains more than one acetate group per molecule and this suggestion was later also confirmed (see below).

The two strong bands at 946 and 979 cm^{-1} are associated with geometrical isomerism. [8,16,18] In the spectra of the all-*trans* compounds CX_2[5,6], CX_4[5], and Aethusin[18], these bands are found at almost exactly the same frequences as in CZ. Moreover, any *cis* compound should show a band in the 650—820 cm^{-1} region. [16,24-28] CZ has no absorption in this region (spectrum run in CS_2). Thus CZ is considered to be a compound of all-*trans* structure.

In the spectrum of CZ no band is found in the region 905—915 cm^{-1} excluding the presence of a vinyl group. [25,26,29] CX_4 that has a vinyl group outside its chromophoric system, shows a band characteristic of the vinyl group at 912 cm^{-1}.

Chemical analyses of CZ. Qualitative elementary analysis showed that no elements other than C, H, and O are present in CZ, and from the quantitative analysis (see p. 1074) the minimal formula was calculated to $C_{21}H_{26}O_4$ and this unit corresponds to a molecular weight of 342.4.

A further proof that CZ is an ester was made by the ferric hydroxamate test. [30-32] *

From the I.R.-analysis (*cf.* above) it was found that CZ contains at least one acetoxy group. On chemical analysis with the lanthanum nitrate-iodine test (*cf.* p. 1074) a strong colour reaction was obtained. This shows that CZ

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* The presence of an anhydride group is excluded since the I.R.-spectrum of CZ shows only *one* carbonyl absorption band (see Fig. 2); anhydrides all show *two* carbonyl bands.

contains acetoxy and/or propionyloxy groups.[33,34] Since, however, the I.R.-spectrum proves that there is no propionyloxy group in CZ (see p. 1070) the above mentioned colour reaction is significant for the presence of at least one acetoxy group in CZ.

The quantitative analysis for O-acetyl was carried out according to Kuhn and Roth [35]. Thus 25.35 % CH_3CO was obtained.

Hydrogenation of CZ and calculation of the molecular weight from the results of the hydrogenation. When hydrogenating CZ, the micromethod according to Clauson-Kaas and Limborg [36] was applied. In testing the apparatus, a series of ten hydrogenations was carried out in ethanol with different amounts of sorbic acid. From the amount of hydrogen absorbed, the molecular weight for sorbic acid was calculated to 112.3 ± 0.3 considering the standard mean error, and to 112.3 ± 1.3 taking the maximal error into account; the relative maximal error was thus found to be 1.2 %. The true molecular weight for sorbic acid is 112.1. As is apparent, the found value, 112.3, is very close to the theoretical value, 112.1, the deviation being only 0.2 %. When hydrogenating a sample of CZ, the experiment was run in tetrahydrofuran *. If besides the chromophoric system no more reducible groups or atoms are present in CZ, *seven* moles of hydrogen would be absorbed (*cf.* p. 1068) and the molecular weight, M, of CZ can be calculated from

$$\mathrm{M} = \frac{W \times 760 \times RT}{(B-b) \times V} \times n_{\pi} \tag{1}$$

where W is the weight of CZ, B the atmospheric pressure, b the vapour pressure of the solvent (tetrahydrofuran) and $n_{\pi} = 7$. *The molecular weight* (*M*) *turned out to be 339.* The maximal error in this determination of M is set to 1.2 %, *cf.* experiment with sorbic acid above, *i.e.* the value is 339 ± 4 **. It is seen that this value of the molecular weight (339) agrees well with the minimal molecular weight as found by combustion analysis (342). ***

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* Tetrahydrofuran and not alcohol was chosen since a possible isolation of the hydrogenated product could have been disturbed if ethanol was present (risk for transesterfication). Attempts were also made to isolate the hydrogenated product but due to the small amount of substance (a few milligrams) that had to be handled the isolation was unsuccessful.

** A change from ethanol to tetrahydrofuran as a solvent (*cf.* text above) cannot possibly change the maximal error 1.2 %, since the vapour pressure values of ethanol [37] and of tetrahydrofuran [38], used in the calculations are sufficiently accurate.

*** An objection that the true molecular weight could be a multiple of the value that was obtained from the hydrogenation of CZ, will be met as follows: Doubling the molecular weight but at the same time keeping the chromophoric system (*cf.* p. 1068) intact means that two hydrogen atoms have to be eliminated. The resulting formula is then equal to twice the empirical formula diminished by two hydrogen atoms ($2 \times C_{21}H_{26}O_4 - 2H$), *i.e.* $C_{42}H_{50}O_8$. The theoretical hydrogen content would then be equal to 7.38 %. The error in the hydrogen determinations by combustion analyses at this laboratory is certainly not greater than 1 %. If the hydrogen value 7.38 % were the correct one, then the error in the hydrogen value found (7.57 %) is equal to 2.6 %. If instead the hydrogen value corresponding to the formula $C_{21}H_{26}O_4$ (7.65 %) were the correct one, then the error is 1.0 %. These facts support strongly that *the true molecular weight is not a multiple of the molecular weight obtained from the hydrogenation.* Considering multiples greater than 2, a still worse agreement with the hydrogen values is obtained. (Owing to shortage of substance no ebullioscopical or cryoscopical determination of the molecular weight could be accomplished.)

If the compound CZ also contained an *isolated* double bond outside the chromophoric system, eight ($n_\pi = 8$) moles of hydrogen would have been absorbed giving a molecular weight of 387 ± 4. This value deviates so strongly from the one that was obtained by the elementary analysis (342) that it is unlikely that the compound contains an isolated double or triple bond.

Determination of the number of acetoxy groups and establishment of the character of the oxygen atoms in CZ. The number of acetoxy groups per molecule CZ was calculated from the O-acetyl analysis (25.35 % CH_3CO, see p. 1075) and from the molecular weight (339) obtained from the hydrogenation experiment.

Thus,

$$n_{\mathrm{Ac}} = \frac{25.35 \times 339}{100 \times 43.05} = 2.0 \qquad (2)$$

where 43.05 is the "molecular" weight of the acetyl group. Evidently *CZ contains two acetoxy groups per molecule.*

The oxygen content by difference in CZ is 18.5 % (*cf.* elementary analysis, p. 1074).

Thus,

$$n_{\mathrm{O}} = \frac{18.5 \times 339}{100 \times 16} = 3.9 \qquad (3)$$

The conclusion is therefore that *CZ contains four oxygen atoms per molecule.* Now, since CZ contains two acetoxy groups, it is obvious that *all four oxygen atoms belong to the two acetoxy groups.*

Calculation of the empirical formula of CZ from the molecular weight, the percentage oxygen, and the percentage acetyl. The empirical formula can be calculated, not only from the elementary analysis, but also from the molecular weight (obtained from the hydrogenation) and the contents of oxygen and acetyl.

If CZ is written as

$$R_1-\underbrace{CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH}_{C_{10}H_6}-R_2$$

the following discussion may be made *:

The sum of R_1 and R_2 includes two acetoxy groups (= $C_4H_6O_4$) and a hydrocarbon residue, which can be written C_xH_{2x}; this unit cannot contain any double or triple bonds (see above). The chromophoric system has the elementary formula $C_{10}H_6$. Consequently, the empirical formula for CZ can be taken as the sum of the three parts:

$$CZ = C_{10}H_6 + C_4H_6O_4 + C_xH_{2x} \qquad (4)$$

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* R_1 and R_2 are placed at the ends of the chromophore since the discussion above (see p. 1068) leads to the conclusion that the chromophoric system is composed of an *unbranched* carbon chain. This view is also supported by the fact that no *vinyl* band can be traced in the I.R.-spectrum (see p. 1070). In this connexion it may be emphasized that among the natural polyenynic compounds hitherto found (about fifty) there is *none* that contains a branched chromophoric chain.[39-43]

Since the molecular weight of CZ was found to be 339 ± 4 (*cf.* p. 1071), we have

$$C_{14}H_{12}O_4 + C_xH_{2x} = 339 \pm 4 \tag{5}$$

or

$$C_xH_{2x} = 95 \pm 4 \tag{6}$$

Supposing $x = 6$, 7, or 8, we obtain

(i) for $x = 6$, $C_6H_{12} = 84.2$
(ii) for $x = 7$, $C_7H_{14} = 98.2$
(iii) for $x = 8$, $C_8H_{16} = 112.2$

Hence, it is obvious that

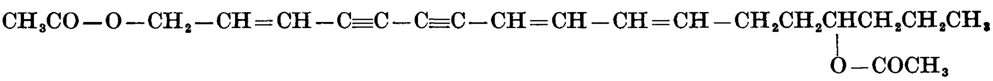
$$C_xH_{2x} = C_7H_{14} \tag{7}$$

and

$$CZ = C_{14}H_{12}O_4 + C_7H_{14} = C_{21}H_{26}O_4 \tag{8}$$

Accordingly the formula for CZ is found to be $C_{21}H_{26}O_4$ and this formula is identical with the one which was found from the elementary analysis alone. For that reason, the composition $C_{21}H_{26}O_4$ for CZ is considered as certain.

It may be observed that the *unknown* diacetate of oenanthotoxin *



$$CH_3CO-O-CH_2-CH=CH-C\equiv C-C\equiv C-CH=CH-CH=CH-CH_2CH_2\underset{\substack{| \\ O-COCH_3}}{CH}CH_2CH_2CH_3$$

must have the following properties in common with CZ: (*a*) empirical formula, $C_{21}H_{26}O_4$, (*b*) U.V.-spectrum, (*c*) chromophoric system, (*d*) all-*trans*-structure [16,17] (*e*) percentage O-acetyl. Such an oenanthotoxin diacetate may be or not be identical with CZ. As to a possible identity of the compounds we cannot, for the present time, make any statement.

EXPERIMENTAL **

Isolation procedure. 9 kg of fresh roots of *Centaurea montana* L. were extracted in the presence of the same weight of anhydrous sodium sulphate with a solvent mixture consisting of chloroform, 21.6 l, and ethanol, 2.4 l. The extraction procedure was performed with 750 g portions of the roots in an electric disintegrator which was situated in a refrigeration room. Atmospheric oxygen was kept away by means of argon gas. The temperature in the extraction mixture was not allowed to exceed 20°C. After filtering, the greyish powder was extracted with chloroform in the same machine. 20 l of chloroform were used for the total amount of this powder. The combined extracts, about 44 l, were freed from solvents by evaporating under reduced pressure (water-bath 20°C). A residue, 42.2 g, was obtained after having dried the substance at 0.1 mm Hg and at a temperature of 20°C. This residue was treated with a mixture of 150 ml cyclohexane and 10 ml carbon tetrachloride. A small insoluble fraction was rejected and the remaining solution, 160 ml, was poured on a column of deactivated aluminium oxide *** moistened with carbon tetrachloride-cyclohexane 1:15 (v/v). Dimensions of the column: 70 by 600 mm. The chroma-

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* Concerning oenanthotoxin, see p. 1069.

** All operations (*e.g.* distillations) were conducted in diffuse light and in argon atmosphere (oxygen-free argon from the AGA Company, Lidingö, Sweden). Only for short periods the temperature was allowed to reach 25°C.

*** Brockmann's aluminium oxide, standardized for chromatographic adsorption analysis; deactivated according to Mancera *et al.*[44]

togram was then developed with carbon tetrachloride-cyclohexane 1:15 (v/v). A broad, diffuse, yellow-brown zone travelled through the column. The main part of this zone was allowed to emerge as a 3.5 l fraction. This fraction had an U.V.-spectrum identical with that of Sörensen's 1,11-tridecadiene-3,5,7,9-tetrayne.[5,8,9] Meanwhile, a slow-moving zone, dark in U.V.-light, had developed, and after having collected the 3.5 l fraction this zone was situated somewhat below the middle of the column. Development was continued with carbon tetrachloride-cyclohexane 1:10 and a pale yellow fraction, about 3 l, still containing the dienetetrayne (see above) was collected. The zone, dark in U.V.-light, was now near the bottom of the column. At this moment the proportion between the components of the eluant was altered from 1:10 to 1:1. When 0.5 l of the new eluant had passed through the column, the actual zone commenced to emerge. Desorption of the zone was complete when a further 3.0 l portion had been collected. This fraction showed the characteristic U.V.-spectrum of Centaur Z, though perturbed by Sörensen's dienetetrayne. The actual fraction was evaporated in *vacuo* to dryness and the residue then taken up in 100 ml of a solvent mixture carbon tetrachloride-cyclohexane 1:10. This solution was chromatographed on aluminium oxide (deactivated, moistened with carbon tetrachloride-cyclohexane 1:10; dimensions of the moistened column: 45 by 400 mm). The chromatogram was then developed with carbon tetrachloride-cyclohexane 1:10. Without altering the composition of the eluant, the fraction corresponding to the U.V.-dark zone (*cf.* above) was collected. This solution, about 2 l, showed by analysis in the ultraviolet a very pure spectrum but still somewhat perturbed by the dienetetrayne. The purification was therefore continued by a third chromatogram. Thus after evaporation to dryness and resolving in 100 ml carbon tetrachloride-cyclohexane 1:10 the chromatographic separation was carried out in the same manner as described above for the second chromatogram. The collected effluent, 1.5 l, gave now an U.V.-spectrum without perturbation from the dienetetrayne. This solution was freed from solvents in *vacuo*. The residue, consisting of a pale yellow crystalline mass, was recrystallized from pentane at −30°C; m.p. 30°C. A second recrystallization from the same solvent raised the melting point to 33°C whereas a third recrystallization did not alter the melting point. The yield of fine colourless needless was 20 mg.

U.V.- and I.R.-spectrophotometrical recordings. The U.V.-spectra were run in a Beckmann spectrophotometer (Model DK-2) with cyclohexane as a solvent. The resulting spectroscopical data for CZ are given in Fig. 1 and Table 1.

The I.R.-spectra were recorded in carbon tetrachloride as well as in carbon disulphide using a Perkin-Elmer instrument (Model 21). Fig. 2 shows the I.R.-spectrum of CZ in carbon tetrachloride: the positions of the actual bands are found on pp. 1069−1070.

Elementary composition. Chemical and infrared analyses of CZ revealed that no other elements than C, H and O are present. *

Quantitative combustion analysis gave a minimal formula equal to $C_{21}H_{26}O_4$. (Found: C 73.9; H 7.57; O (by difference) 18.5. Calc. for $C_{21}H_{26}O_4$: C 73.7; H 7.65; O 18.7.)

Test for ester and acetate. That CZ is an ester was indicated by I.R.-analysis (see p. 1070) as well as by chemical analysis. In the latter case Wille and Rasmussen's [30] micromethod of the ferric hydroxamate test [31,32] was applied. The strong colour reaction obtained revealed the presence of one or more ester groups (*cf.* p. 1070). The infrared analysis had shown that the ester group or groups should consist of at least one acetate group (see p. 1070). The occurrence of the acetoxy group in CZ was demonstrated *chemically* by means of the lanthanum nitrate-iodine test.[33,34] Thus about 1 mg of CZ was heated for one hour at 100°C, together with 3 ml 1 M methanolic potassium hydroxide (methanol: water 7:3, v/v) in a glass ampoule. Most of the methanol was evaporated and the residue neutralized with hydrochloric acid. When adding lanthanum nitrate, iodine, and ammonia to this solution a strong blue colour reaction was obtained. This colour reaction is significant for the acetate ion in the absence of the propionate ion and since CZ does not contain any propionyloxy group [22] (*cf.* p. 1070) the positive colour reaction shows *definitely* the presence of at least one acetoxy group in CZ.

Hydrogenation experiments. The microhydrogenation technique as given by Clauson-Kaas and Limborg [36] was used. The hydrogenation vessel was placed in a thermostatically controlled water-bath and *all* experiments described below were performed at a temperature of 25.10 ± 0.05°C. In order to test the reliability and the accuracy of the apparatus, ten hydrogenations of sorbic acid in ethanol were performed with palladium-

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* Chemical microanalysis performed according to Widmark's [45] technique.

barium sulphate [46] as a catalyst. From eqn. (1) (see p. 1071) the molecular weight (M) was calculated from the weighed amount (W) of the actual compound, the absolute temperature (T), the atmospheric pressure (B), the vapour pressure (b) of the solvent, the volume (V) of hydrogen gas consumed, and the number of C—C-π-bonds (n_π) per molecule. Thus the molecular weight for sorbic acid as a mean from ten experiments was found to be 112.3 ± 0.3 considering the standard mean error, or 112.3 ± 1.3 when the maximal error is taken into account (relative maximal error: 1.2 %).

Due to shortage of substance only one hydrogenation experiment was performed with CZ. The procedure for this hydrogenation was identical with that for the test series except concerning the solvent, which was altered from ethanol to tetrahydrofuran (see footnote *, p. 1071). Volume of tetrahydrofuran: 2 ml, weight of catalyst: 3 mg. Measured quantities: W 2.880 mg, T 298.3°, B 758 mm Hg, and V 1.891 ml. The vapour pressure of tetrahydrofuran (b) at 25.1°C is 173 mm Hg (Klages and Möhler [38]). With these values and with n_π set to 7 (see theoretical part p. 1071), eqn. (1) gives $M = 339$. From the experimental series with sorbic acid (see above) the relative *maximal* error was found to be 1.2 % and for that reason the molecular weight for CZ is taken as 339 ± 4.

Determination of O-acetyl. The quantity of O-acetyl in CZ was determined according to Kuhn and Roth. [35] Thus 1.443 mg CZ gave an amount of acetic acid corresponding to the consumption of 0.848 ml 0.01002 M sodium hydroxide. This result means that CZ contains 25.3_5 % O-acetyl. (Calc. for $C_{17}H_{20}(OOCCH_3)_2$*: CH_3CO 25.14.)

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* *Cf.* p. 1072.

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