PYRROLIZIDINE ALKALOIDS. XIX.*
STRUCTURE OF THE ALKALOID ERUCIFOLINE

P.SEDMERA[a], A.KLÁSEK[b], A.M.DUFFIELD[c] and F.ŠANTAVÝ[b]

[a]*Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6***
[b]*Chemical Institute, Medical Faculty, Palacký University, Olomouc*
[c]*Department of Genetics, School of Medicine, Stanford University, Stanford, Ca. 94305, USA*

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The alkaloid erucifoline $C_{18}H_{23}NO_6$ is assigned structure *VI* on the basis of its chemical reactions, NMR and mass spectral data together with those of its acetyl derivative. The structure of the acid obtained by hydrolysis of erucifoline is discussed.

In the paper[1], we described the isolation of the alkaloid S—C from the plant *Senecio erraticus* BERTH., *ssp. barbaraeifolius* KROCK. In addition, the isolation of the known[2] alkaloids senecionine, integerrimine and othosenine was also described while subsequently seneciphylline was found[3] in this plant. The principal source of the alkaloid S—C proved to be the plant *S. erucifolius* L.[4]. In the plant *S. aegypticus* L., thin-layer chromatography revealed[5] the presence of the alkaloid S—C together with senecionine, othosenine and riddelline. The alkaloid S—C, which we have now named erucifoline, was assigned[4] the empirical formula $C_{18}H_{23}NO_6$. It was shown to contain one active hydrogen and its basic component was identified as retronecine (*I*). Alkaline hydrolysis of erucifoline gave a dicarboxylic acid $C_{10}H_{16}O_7$ which on sublimation yielded a monolactone $C_{10}H_{14}O_6$. Acid hydrolysis of erucifoline yielded a dilactone $C_{10}H_{12}O_5$.

The mother liquors obtained after chromatography of the crude alkaloids from *S. erraticus* gave a small quantity of erucifoline and, therefore, some further measurements were carried out in order to elucidate its structure. The infrared spectrum of erucifoline (in chloroform) exhibits two absorption bands in the carbonyl region at 1742 and 1712 cm^{-1}. These are attributed to a saturated and α,β-unsaturated ester, respectively. The spectra measured in nujol showed one broad band at 1727 to 1717 cm^{-1}. The absorption band of the carbon–carbon double bond appears at 1660 cm^{-1} in chloroform and in nujol. The infrared absorption of the two ester groups (in chloroform) indicated the absence of a hydroxyl group in the α-position of the saturated ester. The presence of such a hydroxyl group would coalesce both carbonyl bands due to the formation of an intramolecular hydrogen bridge between

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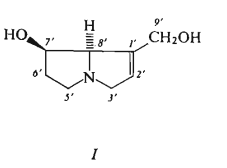
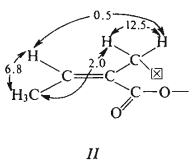
** Present address: Institute of Microbiology, Czechoslovak Academy of Sciences, Prague.

the tertiary hydroxyl and the ester groups[6]. Instead of an intense band in the region of 3000–2800 cm^{-1} (which is usually[6] exhibited by an α-hydroxy ester alkaloid when the spectra are measured in nujol), a sharp band at 3510 cm^{-1} is observed.

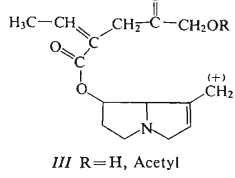
Integration of the NMR spectrum of erucifoline detected 23 protons, one of which is exchangeable for deuterium. The multiplets (two protons) at 5·33 and 4·26 p.p.m., which have a mutual coupling J_{gem} = 12 Hz, are attributable to the protons of a —CH_2—O— group ($H_{(9'u)}$ and $H_{(9'd)}$). The multiplets in the region of 3·2–3·6 p.p.m. correspond to two protons which after addition of tetradeuterio acetic acid are shifted downfield by 0·5 p.p.m. (J = 18 Hz). Consequently, these two protons can be assigned to a methylene group in the α-position to the nitrogen atom ($H_{(3'u)}$ and $H_{(3'd)}$). The two groups of the signals —CH_2—O— and —CH_2—N— show mutual couplings of a magnitude corresponding to homoallylic coupling. They are also coupled to the olefinic proton $H_{(2')}$ which exhibits a large singlet at 6·15 p.p.m. The one proton multiplet at 5·22 p.p.m. couples with two protons in the region of 2·00–2·25 p.p.m. (aliphatic protons at $C_{(6')}$) and with a one proton multiplet at 3·92 p.p.m. Hence, this latter signal is atributable to a proton of the type —CH—O— ($H_{(7')}$). The multiplet at 3·92 p.p.m. is also coupled to the olefinic proton at 6·15 p.p.m. ($H_{(2')}$) and, after addition of tetradeuterio acetic acid, it shifts downfield by 1·13 p.p.m.; thus it is assigned to the proton $H_{(8')}$. The protons in the region of 2·00–2·25 p.p.m. ($H_{(6')}$) also couple with two protons in the region of 3·20–3·40 p.p.m. and 2·50–2·70 p.p.m., which indicates the localization of the remaining two protons at $C_{(5')}$.



I



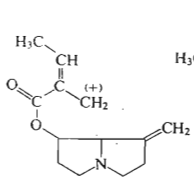
II



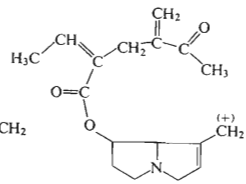
III R=H, Acetyl

The singlet of the tertiary methyl at 1·55 p.p.m. and the doublet of doublets of the olefinic methyl at 1·76 p.p.m. (J = 6·8 and 2·0 Hz) are ascribed to the acidic moiety of erucifoline. The coupling of 6·8 Hz is caused by the geminal olefinic proton at 5·96 p.p.m. which is coupled to the multiplet at 2·38 p.p.m. This multiplet corresponds to the proton which is responsible for the 2·0 Hz splitting of the signal of the olefinic methyl and the 12·5 Hz coupling of the doublet at 3·03 p.p.m. The latter proton also shows a small coupling (<0·5 Hz) to the olefinic proton at 5·96 p.p.m. These findings led to erucifoline containing the partial structure *II*. The magnitude

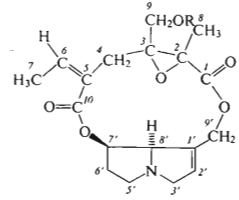
of the chemical shift of the olefinic proton indicates[2] that it is in a *trans* configuration to the ester grouping. This arrangement can also be deduced[7] from the low value of log ε (3·84) of the absorption band at 213 nm in the ultraviolet spectrum of erucifoline. The presence of an ethylidene grouping in erucifoline was also demonstrated from its oxidation by a mixture of osmium tetroxide and periodic acid to acetaldehyde. The remaining AB quartet in the NMR spectrum of erucifoline at 3·62 and 3·93 p.p.m. (J_{AB} = 12·5 Hz) can be ascribed to the methylene protons of the —CH_2OH group. The observed broadening of its high-field member might be accounted for by some long-range couplings.



IV



V



VI R=H
VII R=Acetyl

Acetylation of erucifoline with acetic anhydride in pyridine gave a monoacetyl derivative $C_{20}H_{25}NO_7$. The infrared spectrum of this acetate lacks absorption in the hydroxyl region thus providing confirmatory evidence that erucifoline possesses only one hydroxyl group which is not tertiary[8]. The assignment of the signals in the NMR spectrum of acetyl erucifoline was conducted in the same manner as in the case of erucifoline. A comparison of these two spectra (Table I) was in agreement with the predicted acetylation of the primary hydroxyl group of erucifoline. Moreover, a long-range coupling between the $H_{(4u)}$ and the downfield member of the AB quartet of the group —CH_2OCOCH_3 could be demonstrated, which suggests this group is located at $C_{(3)}$.

The mass spectra of erucifoline and acetylerucifoline exhibit molecular ion peaks at *m/e* 349 ($C_{18}H_{23}NO_6$), and *m/e* 391 ($C_{20}H_{25}NO_7$), respectively. Elimination of COOH (high resolution) from the parent ions of erucifoline and acetylerucifoline is in agreement with their being pyrrolizidine alkaloids[2]. The ion corresponding to M-71 (M − $C_3H_3O_2$ by high resolution) in both spectra can be represented by *III*. The occurrence of this ion in both spectra provides evidence for the localization of the hydroxymethyl, and acetoxymethyl groups, respectively, at $C_{(3)}$. These two spectra also exhibit a peak at *m/e* 220 which corresponds[2] to the fragment *IV*. The mass spectrum of acetylerucifoline contains a peak at *m/e* 288 (M-103). High-resolution

mass spectrometry confirms that this ion arose by expulsion of $CO_2 + CH_3COO$ from the molecular ion and this ion is rationalized in terms of *V*. As anticipated, the ion *V*, M-61, (M − (CO_2 + OH)) appears in the mass spectrum of erucifoline. In the mass region below *m/e* 220, the mass spectra of erucifoline and its acetyl derivative are practically identical and only minor differences in ion abundance are observed. High-resolution mass spectrometry shows that the ions of masses 137, 136, 120, 119, 94, and 93 have an identical composition with those ions observed from the fragmentation of diesters of retronecine[2].

In view of the empirical formula $C_{18}H_{23}NO_6$, erucifoline contains eight degrees of unsaturation (rings and multiple bonds)[9], seven of which have been identified at this stage (two olefinic double bonds, two carbonyl ester groups, two rings in retronecine and one macrocyclic diester ring). One oxygen atom remains unassigned and it might be present as either a carbonyl group or an ether ring (the NMR spectrum does not exhibit any other unascribed signals of protons of the —CH—O—type). In addition, two carbon atoms which do not carry a hydrogen atom but only one tertiary methyl group and one hydroxymethyl group remain to be assigned. The fragment *III* in the mass spectra of erucifoline and acetylerucifoline favours the location of the hydroxymethyl group at $C_{(3)}$, which satisfactorily accounts for the long-range coupling which is observed in connection with the protons of this group in the NMR spectra of *VI* and *VII*. This also indicates that the further substituent

TABLE I

Chemical Shifts (in p.p.m., δ) and Coupling Constants (in Hz) of Erucifoline (*VI*) and Acetylerucifoline (*VII*)

Proton	*VI*	*VII*	Proton	*VI*	*VII*
sp^3—CH_3	1·55	1·56	$H_{(6'u)}$	2·0—2·25	2·1—2·3
sp^2—CH_3	1·76	1·74	$H_{(6'd)}$	2·0—2·25	2·1—2·3
$H_{(4d)}$	3·03	2·83	$H_{(7')}$	5·22	5·22
$H_{(4u)}$	2·38	2·45	$H_{(8')}$	3·92	3·92
$H_{(6)}$	5·96	5·80	$H_{(9'u)}$	4·26	4·32
—CH_2O— d	3·93	4·31	$H_{(9'd)}$	5·33	5·29
—CH_2O— u	3·62	4·14	$J_{6,Me}$	6·8	7·2
CH_3CO	—	2·09	$J_{4u,Me}$	2·0	1·8
$H_{(2')}$	6·15	6·05	$J_{4d,Me}$	lrc[a]	lrc[a]
$H_{(3'u)}$	3·43	3·47	$J_{4u,4d}$	14·0	14·8
$H_{(3'd)}$	4·26	4·35	$J_{gem(CH_2O)}$	12·5	12·5
$H_{(5'u)}$	2·5—2·7	2·5—2·7	$J_{4u,6}$	0·8	1·3
$H_{(5'd)}$	3·2—3·4	3·2—3·4	$J_{4d,6}$	lrc[a]	0·5

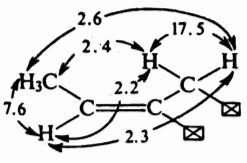
[a] Long-range coupling.

at the quaternary atom $C_{(3)}$ is not a tertiary methyl group but an oxygen atom. This finding excludes the alternatives which take into consideration the presence of a carbonyl group. On the basis of these conclusions, an epoxide ring between $C_{(2)}$ and $C_{(3)}$ is assumed, which leads to the formulae *VI* and *VII* for erucifoline and its acetyl derivative.

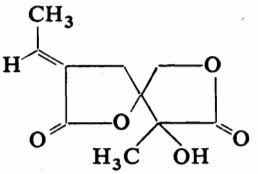
The NMR spectrum of the dilactone of erucifolinecic acid[4] $C_{10}H_{12}O_5$ exhibits an exchangeable singlet of the tertiary hydroxy group at 6·22 p.p.m., a singlet of the tertiary methyl group at 1·31 p.p.m., a doublet of triplets of the olefinic methyl at 2·07 p.p.m., a multiplet of the olefinic proton at 6·38 p.p.m., two multiplets of aliphatic protons at 2·78 and 3·03 p.p.m. (in the allylic position to the double bond), and a two-proton singlet at 4·40 p.p.m. which is ascribed to the protons of the —CH_2—O— group. Double resonance revealed couplings which lead to the formulation of the partial structure *VIII*. From the NMR spectrum, the formulae *IX* and *X* have been inferred for the dilactone. In the infrared spectrum (nujol) of the dilactone, the intense band at 1790 – 1778 cm^{-1} might be assigned (on the basis of a comparison with the infrared spectrum of the lactone of senecic acid[2] (1737 cm^{-1})) to an α,β-unsaturated γ-lactone; consequently structure *IX* is preferred. The *trans*-arrangement of the methyl group to the lactone carbonyl can be inferred from the high value of log ε (4·14) for the absorption band at 218 nm in the ultraviolet spectrum of the dilactone.

Erucifolinecic acid $C_{10}H_{16}O_7$ arises by alkaline hydrolysis[4] of erucifoline (*VI*). The NMR spectrum of its monolactone $C_{10}H_{14}O_6$ exhibits signals of twelve protons: a singlet of a tertiary methyl group at 1·39 p.p.m., a doublet of a secondary methyl group (J = 6·2 Hz) at 1·26 p.p.m. whose methine proton is located at 3·73 p.p.m. (a quartet), the one-proton doublets at 2·03 (J = 11·2 Hz), 2·57 (J = 5·5 Hz) and 3·56 p.p.m. (J = 11·5 Hz), and the multiplets at 2·63 (J = 5·5, 11·2 and 2·5 Hz) and 4·23 p.p.m. (J = 11·5 and 2·5 Hz). The remaining two protons constitute a component of the hydroxyl and the carboxyl group (demonstrated by the preparation of a methyl ester and acetate). Contrary to erucifoline (*VI*), erucifolinecic acid does not possess an ethylidene grouping. The chemical shift of the methine proton of the secondary methyl group indicates that it is a proton of the —CH—O— type which could arise by the addition of a hydroxyl group to a double bond. The proposed structure *XI* for erucifolinecic acid is the only one which conforms with the fact that this acid does not give[4] a positive reaction with periodic acid as would be required by structures containing vicinal hydroxyl groups. Consequently, on alkaline hydrolysis of erucifoline (*VI*), a hydroxyl group at $C_{(3)}$ (arising by opening of the epoxide ring) is added to the $C_{(5)}-C_{(6)}$ double bond. Lactonization of erucifolinecic acid (*XI*) then leads to closure of the six-membered ring between the primary hydroxyl group and the carboxyl $C_{(10)}$ yielding *XII*. The assignment of structure *XII* to the monolactone of erucifolinecic acid is also supported by the fact that after addition of trichloroacetyl isocyanate to the solution during measurement of the NMR spectrum

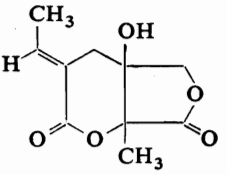
of the monolactone, the signal of the tertiary methyl is shifted by 0·17 p.p.m. downfield[10].



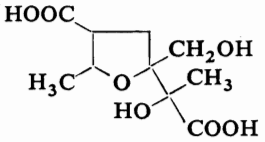
VIII



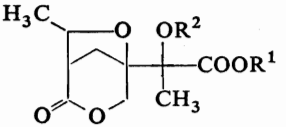
IX



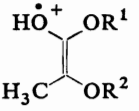
X



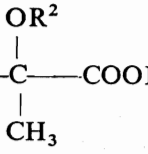
XI

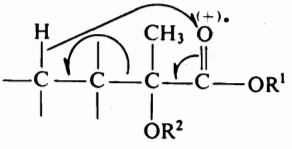


XII; $R^1 = R^2 = H$
XIII; $R^1 = CH_3$, $R^2 = H$
XIV; $R^1 = CH_3$, R^2 = Acetyl



XV

The ion at *m/e* 90 in the mass spectrum of the monolactone *XII* derived from erucifolinecic acid is of importance for the confirmation of structure *XII*. By high-resolution mass spectrometry its composition was determined to be $C_3H_6O_3$ and it can be formally designated as *XV* ($R^1 = R^2 = H$) the product ion from a McLafferty rearrangement of the unit *XVI* ($R^1 = R^2 = H$). In the mass spectrum of the ester lactone *XIII*, an analogous fragment *XV* ($R^1 = CH_3$, $R^2 = H$) appears at *m/e* 104 and, in the spectrum of the acetylated ester lactone *XIV*, the fragment *XV* ($R^1 = CH_3$, R^2 = acetyl) occurs at *m/e* 146 which by loss of ketene (verified by a metastable peak) yields an ion of mass 104. An abundant ion of mass 141 is observed in the mass spectra of *XII*, *XIII* and *XIV* and this fragment could arise from the expulsion of the  unit in each instance. Chemical confirmation of the structure *XII* for the monolactone of erucifolinecic acid was not possible due to insufficient material being available.



XVI

EXPERIMENTAL

Melting points were measured on a Kofler block and are uncorrected. The IR spectra were measured on an Infrascan (Hilger, England), the UV spectra on a Unicam SP-700 (England), the mass spectra on a G.E./A.E.I. MS-9 (U.S.A.), and the NMR spectra on a Varian HA-100 (U.S.A.) using hexamethyldisiloxane (HMDS) as an internal standard. The chemical shifts are given as $\delta_{(TMS)}$ values ($\delta_{HMDS} = 0.06$ p.p.m.). For the CD values of erucifoline see paper[11].

Acetylerucifoline (*VII*)

To a solution of 55 mg of resublimed erucifoline (*VI*) (m.p. 195—197°C) in 1 ml of pyridine, acetic anhydride (2 ml) was added and the mixture left standing for 2 days at room temperature. The reaction mixture was evaporated to dryness *in vacuo* and the residue dissolved in 5% citric acid. The solution was washed three times with ether, made alkaline with ammonia, extracted five times with chloroform, dried over anhydrous sodium sulphate, and the solvent removed by distillation. The residue was recrystallized from a mixture of benzene–cyclohexane to afford acetylerucifoline (*VII*) as needles, m.p. 127—129°C. High-resolution mass spectrometry established the empirical formula $C_{20}H_{25}NO_7$. Thin-layer chromatography of acetylerucifoline (*VII*) on silica gel G (solvent system benzene–ethyl acetate–diethylamine 7 : 2 : 1) gave a spot of R_F 0·46 (erucifoline 0·38) after spraying with Dragendorff reagent.

Oxidation of Erucifoline (*VI*)

To a suspension of 78 mg of erucifoline (*VI*) in 10 ml of water, was added a solution of 500 mg of periodic acid and 20 mg of osmium tetroxide in 10 ml of water. The reaction mixture was left standing for 3 h at room temperature. After addition of crystalline ferric sulphate (10 g), the mixture was left standing for 6 hours, heated to 100°C, and a stream of nitrogen bubbled through the reaction mixture. The stream of gas was then passed into 10 ml of a 5% solution of dimedone in a mixture of water–ethanol (1 : 1). After 24 h, the precipitated crystals were filtered off, dissolved in 2 ml of acetic acid, and the solution refluxed for 6 hours. After cooling and addition of 50 ml of water, the precipitate was dried, washed with 7 ml of 1M-NaOH, and recrystallized from aqueous ethanol to yield platelets of m.p. 174—175°C which did not show a depression on admixture with the anhydrodimedone derivative of acetaldehyde.

Ester Lactone of Erucifolinecic Acid (*XIII*)

To a solution of 120 mg of the monolactone of erucifolinecic acid[4] (*XII*) in 3 ml of methanol, excess of an ethereal solution of diazomethane was added. After standing for 30 min at room temperature, the reaction mixture was evaporated to dryness *in vacuo*. Crystallization of the product from ether–light petroleum gave prisms of m.p. 79—82°C. The empirical formula $C_{11}H_{16}O_6$ was established by high-resolution mass spectrometry.

Acetylated Ester Lactone of Erucifolinecic Acid (*XIV*)

To a solution of 70 mg of the ester lactone of erucifolinecic acid (*XIII*) in 1 ml of pyridine was added 1 ml of acetic anhydride, the reaction mixture left standing for 3 days at room temperature, and then evaporated to dryness. The residue was dissolved in ether, the solution washed with water and dried over anhydrous sodium sulphate. After removal of the solvent, the residue was crystallized from a mixture of ether–light petroleum to give needles of m.p. 138—140°C. The empirical formula $C_{13}H_{18}O_7$ was determined by high-resolution mass spectrometry.

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