Chemical Investigation of Anticharis Linearis Hochst, Part-II (Structure of Linearin and Linearoside)

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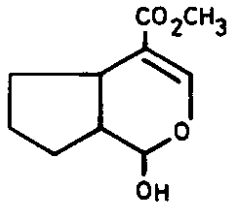
Summary: Linearin and linearoside have been assigned the structures as shown by fig-1 and fig-2 respectively, mainly based on spectral interpretation and comparison with other known iridoids. Linearoside is a β-D-glucoside of linearin as confirmed by enzymatic hydrolysis.

In the previous article[1], the isolation and characterisation of linearin and linearoside alongwith two known compounds i.e. mannitol and triacontane have been reported. This paper deals with the structure elucidation of linearin (fig. 1) and linearoside (fig. 2) on the basis of spectral studies and their close relationship with genipin[2], loganin[3], barlerin[4] and other naturally occuring iridoids.

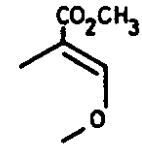
Linearin (Fig. 1) gives the molecular ion peak at m/e 228 corresponding to $C_{11}H_{16}O_5$ and contains two acetylable hydroxyl groups[1]. It responds to hydroxamic acid test and does not form any derivative of keto group. The presence of an ester group is also supported by the signal at m/e 59 in the mass spectrum. Four oxygen atoms are thus accounted for. The fifth oxygen may be present as an ether linkage perticularly since it does not respond to Kuhn-Roth test for methoxy group.

The presence of an enol-ether group conjugated with the carbomethoxyl function (-O-C=C-COOR) is indicated by the bands in the infrared at 1690 cm^{-1} (-CO_2 CH_3) and 1625 cm^{-1} (-C=C-O-) and by the absorption in the ultraviolet at 238nm. (ε 11000). Linearin, therefore, appears to possess chromophore (A).



The difficulty experienced in hydrogenating the double bond is also in accord with this chromophore since this difficulty was encountered with loganin[3] which possesses a similar structure. This suggests that there is no other double bond apart from that in the chromophore. The reduction of Fehling solution by linearin (Fig. 1) shows the presence of hemiacetal group[5] (-C=CH-O-C-OH), which also supplements the presence of chromophore (A) and position of one of the hydroxyl groups.

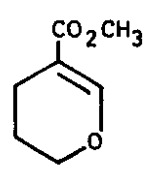
(A)



The presence of a pyran ring finds support from a comparison of the UV spectrum of linearin with the spectra of the closely related iridoids e.g. genipin[2], loganin[3], barlerin[4], lamalbid[6], and morroniside[7] (Table 1). The presence of a furan ring is excluded since it would be expected to absorb at 251 nm, rather than the values of 234 -238 nm[8]. Therefore, the following skeleton of linearin is indicated, which is also corroborated by the evolution of carbon dioxide on acid hydrolysis.

The 60 MHz nmr spectrum of linearin di-acetate in $CDCl_3$ reproduced in fig-3, is also in full accord with the proposed structure. The singlet at 6.25 τ is due to the ester methyl and the lowfield olefinic proton at C_3 (2.61 τ) can only be attributed to -O-CH=C- as in lamalbid, morroniside, genipin, gardenoside[9] (Table 1). and its doublet nature must be due to allylic coupling. Chromophore (A) is thus supported. The other low field proton at 3.55 τ is not vinylic as it is too low to be due merely to the group AcO-Ċ-H and must therefore be due to the methine proton in AcO-CH-O-. Its coupling requires a vicinal-Ċ-H group, which must be that resonating at 7.35 τ. The latter, in turn, is also coupled with a -CH group which the line perturbation shows to be that resonating at 6.87 τ. Therefore the chromophore (A) can be expanded to chromophore (B): it is also supported by the presence of the mass ion m/e 157.

(B)



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The signal at 8.46 τ for C-CH_3 shows that it is attached to a tertiary carbon (C-8) in a cyclopentane ring similar to barlerin[4] and lamalbid[6] (Table 1). This would mean that the second hydroxyl group in linearin is a tertiary one.

The structure of linearin then could be completed as in fig 1.

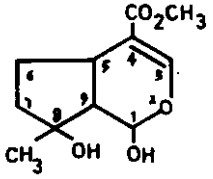

Fig 1

The fragmentation pattern (Table 2) of linearin, as shown by the mass spectrum, further confirms the proposed structure. It shows a molecular ion peak at m/e 185 ($C_9H_{13}O_4^+$, 74%). This ion then undergoes two successive losses of 14 mass units for -CH_2 groups giving rise to m/e 171 ($C_8H_{11}O_4^+$, 24%) and m/e 157 ($C_7H_9O_4^+$, 10.93%). The latter loses a molecule of water to give m/e 139 ($C_7H_7O_3^+$, 82.18%) which disintegrates further to m/e 126 ($C_6H_6O_3^+$, 54.8%) followed by m/e 67 ($4H_3O^+$, 24%). By another mode of fragmentation the molecular ion (m/e 228) loses two molcules of water successively to give peaks at m/e 210 ($C_{11}H_{14}O_4^+$, 10.98%) and m/e 192 ($C_{11}H_{12}O_3^+$, 10.85%). The latter then shows a loss of 84 mass units (-$C_4H_4O_2$) giving rise to ionic species at 108 ($C_7H_8O^+$, 37%) which further undergoes fragmentation to give peaks at m/e 93 ($C_6H_5O^+$, 31.5%) followed by m/e 65 ($C_5H_5^+$, 18.1%) showing loss of methyl group and carbon monoxide respectively. A peak at m/e 195 ($C_{10}H_{11}O_4^+$, 9.85%) also appears due to loss of a methyl group from m/e 210, which breaks up into m/e 153 the base peak ($C_8H_9O_3^+$, 100%) and m/e 135 ($C_8H_7O_2^+$, 42.2%) losing a -$COCH_2$ group followed by a molecule of water. Other prominent peaks are m/e (%): 182(23.29), 178(60.3), 154(70.0), 125(65.75), 111(60.1), 97(61.64), 81(45.2), 59(23.8), 43(65.9), 39(37.23), 31(31.5), 28(54.28), 18(41.6) and 15(24.0). The peaks at m/e 15, 28, 31, 43 and 59 together indicate a methylester group representing -CH_3, -CO, -OCH_3, -CH_3CO and -$COOCH_3$ respectively. The intense peak at m/e 43 (65.90%) cannot, however, be entirely due to ester group since it is known that the esters with moieties heavier than acetates do not give strong peaks[10] for m/e 43. The greater part of strength of this peak is probably contributed by some other moiety. This points to a methyl and hydroxyl groups attached to an active carbon atom, which must therefore be a tertiary one i.e. C-8 in linearin (Fig. -1) and is confirmed by its fragmentation.

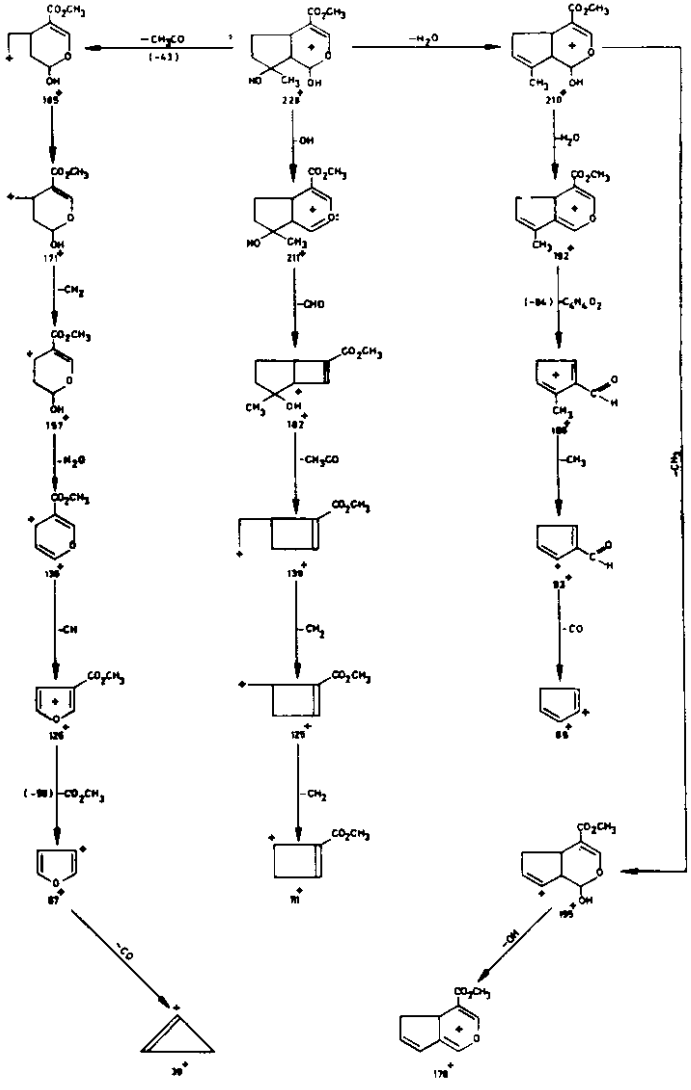

Chart 2.

Linearoside (Fig- 2) corresponds to the molecular formula $C_{17}H_{26}O_{10}$. The IR bands at 1690 cm^{-1}, 1640 cm^{-1} and the UV absorption at 236 nm (ϵ 5000) indicate the presence of enol-ether group conjugated with carbomethoxy function (-O-C=C-COOR). The nmr of its tetra-acetate gives signals at 6.21 τ (-$COOCH_3$), 2.59 τ (O-CH=C-), 7.87 τ (acetate), 7.97 τ (acetate) and 8.48 τ (methyl), similar to the di-acetate of linearin. The signal of the anomeric proton at C-1 (4.20 τ) shows that the glucose is in β-configuration. This is confirmed by the production of linearin (Fig-1) and glucose on hydrolysis with β-emulsin. Hence linearoside is β-D-glucoside of linearin.

Linearoside forms a tetra-acetate and does not produce a penta-acetate even under drastic conditions, which further proves that the additional hydroxyl group (at C-8) is a tertiary one, since other iridoids e.g. gardenoside[9], gluroside[11], with hydroxyl groups in similar positions behave in the same manner.

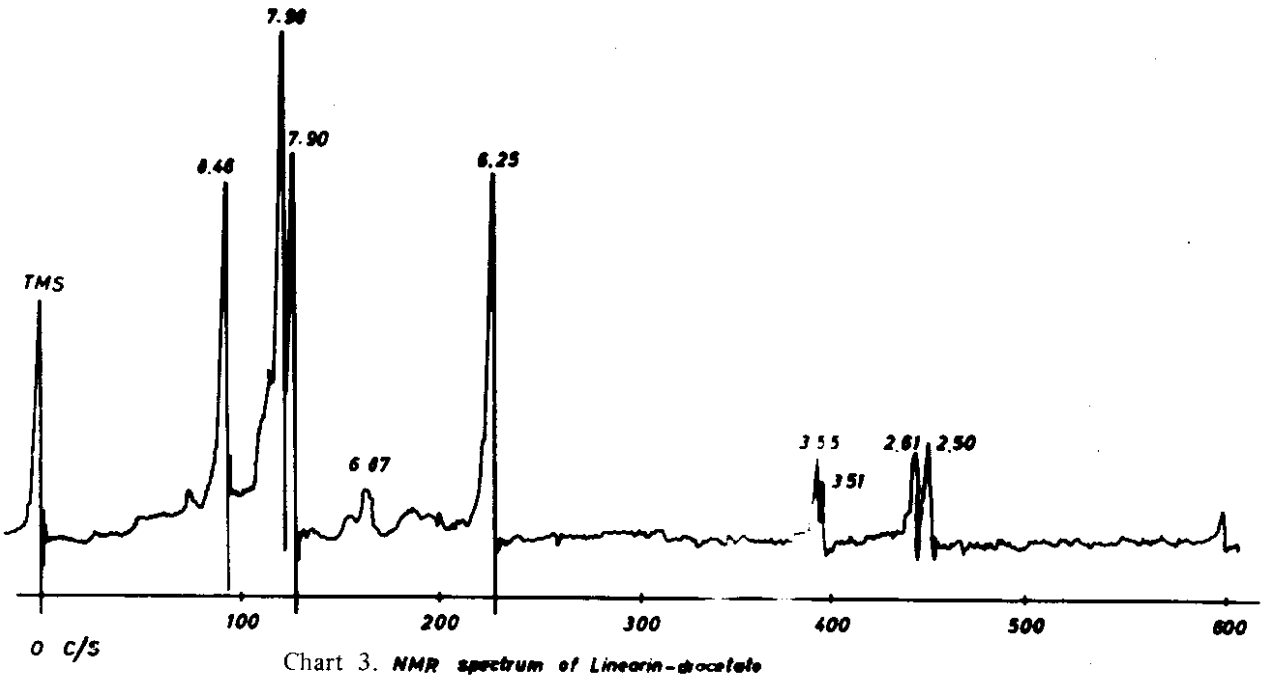

Chart 3. NMR spectrum of Linearin-diacetate

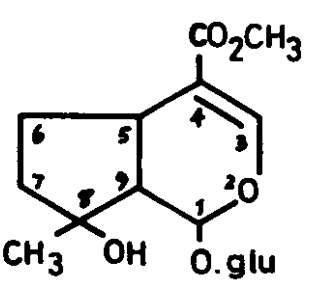

Fig. 2

Table. 1.

	Compound	U.V. (nm.)	N. M. R. (τ) $-CO_2CH_3$	$-C=C-O-$	$C-CH_3$ (C-8)	C_1 - H
1.	Genipin[2]	240	6.27	2.48	–	4.14
2.	Barlerinaglycone[4]	235	6.28	2.39	8.50	–
3.	Lamalbid[6]	235	6.26	2.55	8.80	–
4.	Morroniside[7]	236	6.27	2.60	–	–
5.	Gardenoside[9]	237	6.25	2.55	–	4.18
6.	Asperuloside[12]	234.5	6.21	2.80	–	4.32
7.	Nyctathoside[13]	237	6.18	2.45	–	4.65
8.	Linearin	238	6.34	2.66	8.66	–
9.	Linearin-di-acetate	–	6.25	2.61	8.46	–
10.	Linearoside	236	6.29	2.51	8.79	4.61
11.	Linearoside-tetra-acetate	–	6.21	2.59	8.48	4.20

Experimental

The m.ps. are uncorrected and were obtained on Fisher-Johns hot stage melting point apparatus. The ultraviolet spectra were recorded on a Beckman Model DB spectrophotometer in 1 cm. silica cells using doubly distilled water as the solvent. The infrared spectra were taken on Perkin-Elmer Infracord Model 137 infrared spectrophotometer. The nmr spectra were obtained on a Varian A-60 spectrometer using TMS as the standard. The mass spectra were recorded on an A.E.I. MS-902 mass spectrometer.

Hydrolysis of Linearoside:

Linearoside (0.5 gm, 1.3 mmol) and 2N H_2SO_4 (15 ml.) were warmed on a water bath when a black substance settled down. It was filtered and from the aqueous solution; an osazone (0.13 g, 28.2%) was prepared by the usual method. The product was identified by m.p. & mixed m.p. with an authentic sample as the glucosazone. T.L.C. showed it to be a single compound.

Enzymatic hydrolysis of linearoside:

The hydrolysis of linearoside (0.5g, 1.3 mmol) with β-emulsin (from sweet almonds) was carried out by usual procedure.[14] The reaction mixture was then extracted with chloroform several times. The solvent was removed and the residue crystallised form benzene into needles (0.0822g, 35%) m.p. 122-23°C. It was identified as linearin by mixed m.p. and I.R. Spectrum.

Acknowledgements

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