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CHEMICAL INVESTIGATION OF ANTICHARIS LINEARIS HOCHST. PART I

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From *Anticharis linearis* Hochst, two hydroxy lactones $C_{11}H_{16}O_5$, m.p. 123–24°C, $C_{17}H_{26}O_{10}$, m.p. 182–83°C, provisionally named as linearin and linearoside, respectively, have been isolated. A crystalline polyhydroxy compound and an aliphatic hydrocarbon identified as mannitol and triacontane, respectively, have aslo been isolated.

The plant *Anticharis linearis* Hochst (Scrophulariaceae) grows on the hills of Thanabulakhan (District Dadu) and is also found scattered in the Plains of Jamshoro and Khairpur in the province of Sind. It is used by the locals as a cure for diabetes mellitus. The plant is an annual herb with linear leaves and violet flowers flourishing in the month of September–October and not exceeding a maximum height of six inches to 1 ft. It is usually available after summer rainfall.

It will be interesting to note that a number of members of Scrophulariceae family have been reported in indiginous medicine to be useful in the treatment of diabetes.[1] Chemical examination of the above plant was undertaken to correlate the results with the investigations on the other two members of the same family being simultaneously carried out in these laboratories.

Preliminary pharmacological studies of the above plant revealed that when water extract of the semidried plant was injected into rats and dogs, marked variations in blood pressure were observed and in few cases stimulation of heart was also noticed.[2] On the other hand, preliminary chemical examination revealed that the plant is fairly rich as far as the glycosides are concerned. Some other active principles have also been isolated. Many members of this family have been found to contain glycosides. R. Jaretlzky and H. Ulrici[3] investigated about sixty species of the family and identified cardioactive glucosides in many members.

The scheme for the isolation of the compounds is given in the chart.

Linearin was obtained from the watersoluble portion of the chloroform fraction, on passing through a column of alumina with benzene as the solvent. It reduced dilute potassium permangnate solution and decolorised bromine in acetic acid. It had m.p. 123–24°C and analysed for $C_{11}H_{16}O_5$. Its IR spectrum (Fig. 1) revealed the presence of unsaturation hydroxy and keto groups. No derivative of keto group could be prepared. The UV light absorption, however was typical of α,β-unsaturated carbonyl group. It gives positive hydroxamic acid test indicating a lactonic or ester function and liberates carbon dioxide on hydrolysis with dil. H_2SO_4. These tests show the presence of lactone group. The main peaks in IR and UV were comparable with those of β-hydroxy butenolides (tetronic acids) which are also known to shed off carbon dioxide on hydrolysis with dilute acids.[5]

The ethyl acetate fraction, on purification by passing through a column of alumina with ethyl acetate as the eluent, gave needles of linearoside which melted at 182–83°C. It analysed for $C_{17}H_{26}O_{10}$ and showed peaks of keto unsaturation and hydroxyl groups in the IR region (Fig. 3). It formed a crystalline tetracetate, m.p. 136–38°C and tetrabenzoate, m.p. 156–58°C. It evolved carbon dioxide on heating at 250–80°C and the residue crystallised from benzene m.p. 165–68°C. It showed no carbonyl absorption in the IR spectrum. On chromic acid oxidation linearin was obtained which shows that linearoside is structurally related to linearin.

The ethyl alcohol fraction on concentration and cooling gave white needles, m.p. 163–64°C. Its IR spectrum was superimposible on that of D-mannitol. Its mixed m.p. with an authentic sample and mixed m.p. (123–25°C) of its acetate with that of D-mannitol acetate confirmed it as D-mannitol.

Besides mannitol, glucose and mannose were present in the alcohol fraction when examined by paper chromatography. D-Glucose and D-mannose were also isolated as phenyl hydrazone derivatives.

Two more hydroxy compounds named as anticharol, m.p. 296–98°C and linearol, b.p. 148–49°C/0.2 mm, have been isolated from the water-insoluble residue of the alcoholic extract. Their acetate derivatives, anticharol acetate, m.p.

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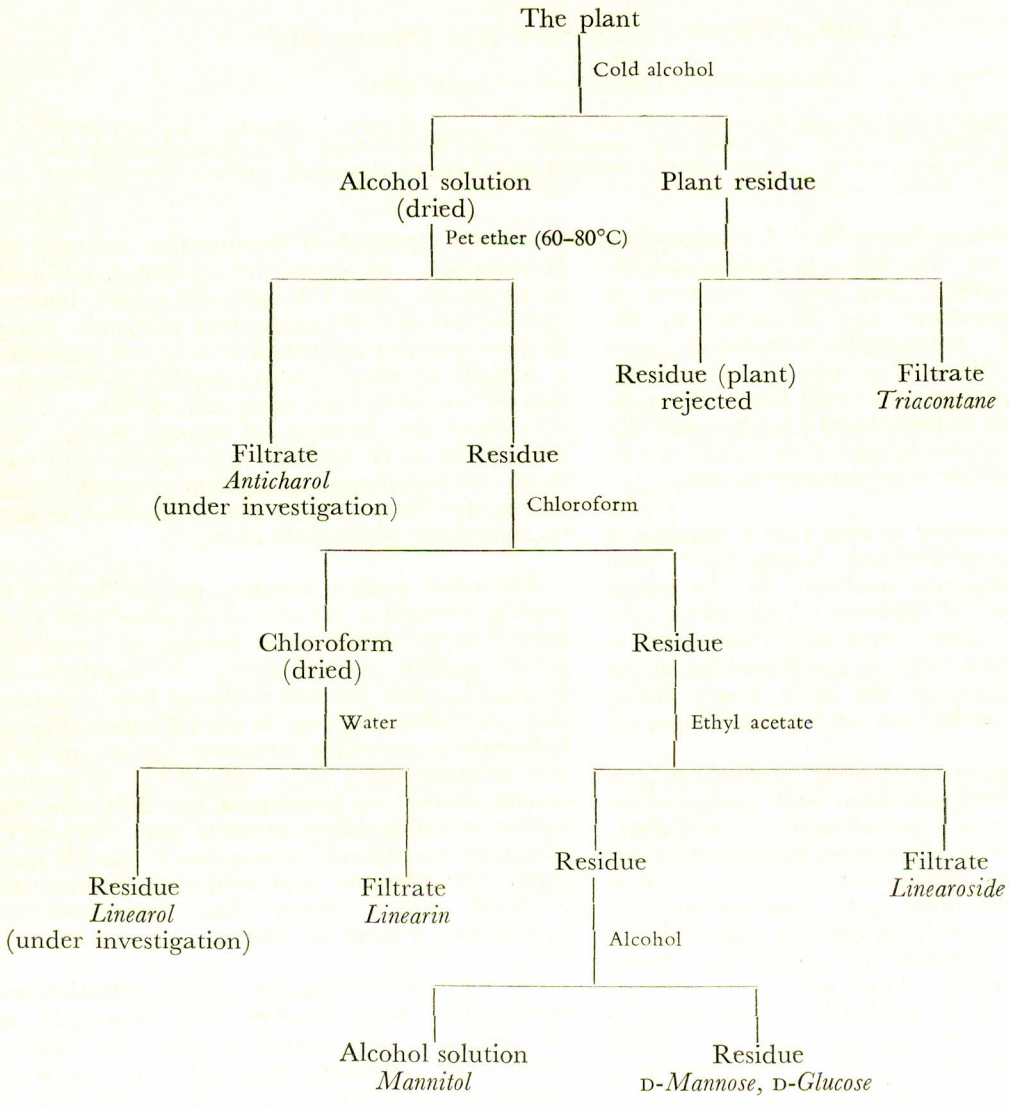
The plant
Cold alcohol
Alcohol solution (dried)
Plant residue
Pet ether (60–80°C)
Residue (plant) rejected
Filtrate *Triacontane*
Filtrate *Anticharol* (under investigation)
Residue
Chloroform
Chloroform (dried)
Residue
Water
Ethyl acetate
Residue *Linearol* (under investigation)
Filtrate *Linearin*
Residue
Filtrate *Linearoside*
Alcohol
Alcohol solution *Mannitol*
Residue D-*Mannose*, D-*Glucose*

157–59°C, and linearol acetate, m.p. 124–26°C, have also been prepared and will be reported latter.

The benzene extract of the plant on concentrating and passing through a column of alumina, yielded triacontane $C_{30}H_{62}$, m.p. 66–67°C. Its IR spectrum was exactly similar to that of triacontane.

Experimental

All the m.ps are uncorrected and were taken on Fisher-Johns hot stage melting point apparatus. The IR spectra were taken on Beckman, IR spectrophotometer and UV spectra on Beckman DB spectrophotometer. The microanalysis was carried out by microanalytical section P.C.S.I.R. Laboratories, Karachi and by Alfred Bernhardt Microanalytical Laboratories, West Germany. The mol. wts were determined by crysocopic method in our laboratories and further confirmed by mass spectra.

Isolation of Linearin.—The plant *Anticharis linearis* (50 kg) was kept immersed in cold alcohol for a fortnight. The alcohol was removed by distillation and the extract was dried at 80°C. The semisolid residue thus obtained was heated under reflux with petroleum ether (60–80°) to remove chlorophyll and fats. It was then extracted with chloroform exhaustively. The chloroform extract was washed with hot water successively and the water-soluble portion, after drying on a water bath, was dissolved in a minimum quantity of alcohol and adsorbed on neutral alumina (E. Merck). It was eluted with benzene and 10 fractions of 100 ml each were collected. No crystalline product separated from the first four fractions whereas from the following fractions needle-like crystals separated after a week at the room temperature. The crystals from different fractions were combined and twice recrystallised from ether to give linearin m.p. 123–124°C, (19.8 g, 0.04%). (Found: C, 57.9; H, 7.0. $C_{11}H_{16}O_5$ requires: C, 57.8; H, 7.0%), $[\alpha]^{30}_D$ −13.5, UV max. 238 mμ (ε 11000).

Linearin was soluble in chloroform, ether, ethanol, methanol, water and pyridine. It was sparingly soluble in benzene and insoluble in

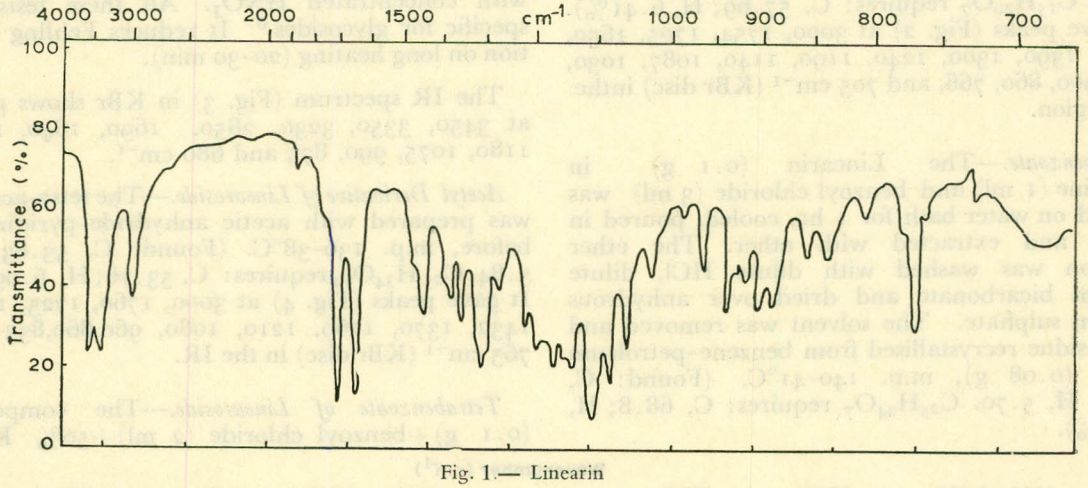

Fig. 1.— Linearin

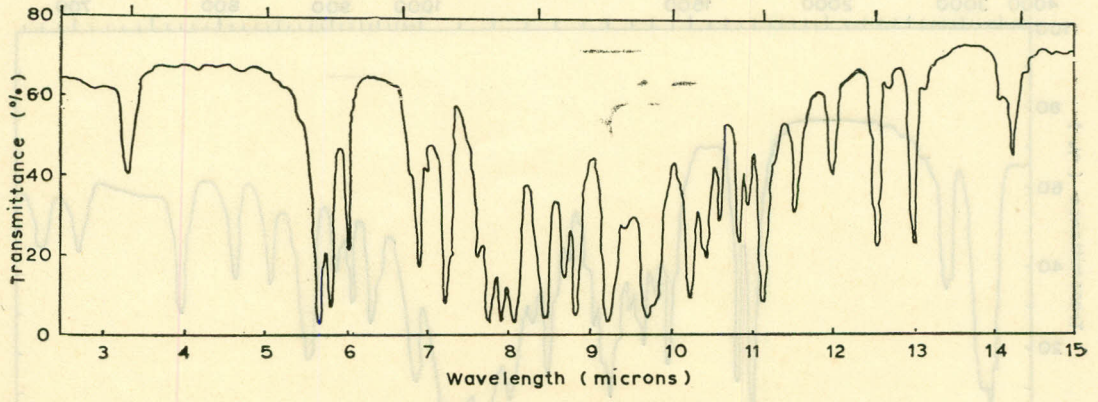

Fig. 2.— Linearin acetate (KBr disc)

petroleum ether. It decolorised dilute potassium permang nate solution and bromine in glacial acetic acid. It reduces Benedict's and Fehling solutions and gives positive test with triphenyltetrazolium chloride indicating a α-ketol group.[4] It gives positive hydroxamic test indicating the presence of ester or lactone group. It evolves carbon dioxide on hydrolysis with 2N H_2SO_4 which is characteristic of β-hydroxy butenolides (tetronic acids).[5]

IR spectrum (Fig. 1) showed peaks at 35000 (–OH group), 3350 (–OH group), 2950, 1690 (ester or lactone), 1625 (unsaturation), 1475, 1300, 1105, 1070, 1050, 960, 930, 835, 805 and 765 cm^{-1} (KBr disc).

Diacetate of Linearin.—The compound (0.1g) in pyridine and acetic anhydride (2.0 ml) was heated for 2 hr on steam bath and cooled in ice. The diacetate formed was successively washed with $NaHCO_3$ solution, dilute HCl and H_2O. It was taken in methanol, charcoaled and recrystallised from methanol–water to give fine needles (0.03 g), m.p. 106–7°C. (Found: C, 58.28; H, 6.21. $C_{15}H_{20}O_7$ requires: C, 57.69; H, 6.41%). It gave peaks (Fig. 2) at 3000, 1754, 1705, 1650, 1450, 1390, 1300, 1240, 1190, 1140, 1087, 1030, 960, 900, 860, 766, and 705 cm^{-1} (KBr disc) inthe IR region.

Dibenzoate.—The Linearin (0.1 g) in pyridine (1 ml) and benzoyl chloride (3 ml) was heated on water bath for 1 hr, cooled, poured in water and extracted with ether. The ether solution was washed with dilute HCl, dilute sodium bicarbonate and dried over anhydrous sodium sulphate. The solvent was removed and the residue recrystallised from benzene–petroleum ether (0.08 g), m.p. 140–41°C. (Found: C, 69.0; H, 5.70. $C_{25}H_{24}O_7$ requires: C, 68.8; H, 5.55%).

Isolation of Linearoside.—The cold dried alcoholic extract from the previous experiment (after the chloroform extraction) was heated under reflux with ethyl acetate several times. The solvent was removed and the residue dissolved in alcohol and then adsorbed on acid alumina (E. Merck). The column was eluted with ethyl acetate. The first fractions (about 500 ml) did not give any crystalline substance but from the following fractions the compound crystallised out on scratching the sides of the flask. It was recrystallised twice from ethyl acetate in the same way when needles of linearoside separated which melted at 182–83°C (23 g, 0.056%). Found: C, 52.14; H, 6.39. $C_{17}H_{26}O_{10}$ requires: C, 52.30; H, 6.67, $[\alpha]^{30}_D$ −110° (5% EtOH). The UV max. at 236 mμ (ε5000).

It is freely soluble in water, ethanol, moderately so in ethyl acetate and acetone, and insoluble in chloroform, benzene, ether and petroleum ether (60–80°C). It gives positive tests for unsaturation and ester group. It produces red colour with concentrated sulphuric acid, violet colour with concentrated HCl and phenol, and a yellow colour with concentrated HNO_3. All these tests are specific for glycosides.[6] It reduces Fehling solution on long heating (20–30 min).

The IR spectrum (Fig. 3) in KBr shows peaks at 3450, 3350, 3230, 2850, 1690, 1640, 1300, 1180, 1075, 990, 875 and 680 cm^{-1}.

Acetyl Derivative of Linearoside.—The tetra-acetate was prepared with acetic anhydride–pyridine as before, m.p. 136–38°C. (Found: C, 53.83, H, 5.84. $C_{25}H_{34}O_{14}$ requires: C, 53.76; H, 6.09%). It gave peaks (Fig. 4) at 3000, 1760, 1725, 1650, 1432, 1370, 1260, 1210, 1080, 960,860,833 and 765 cm^{-1} (KBr disc) in the IR.

Tetrabenzoate of Linearoside.—The compound (0.1 g)+benzoyl chloride (2 ml)+50% KOH

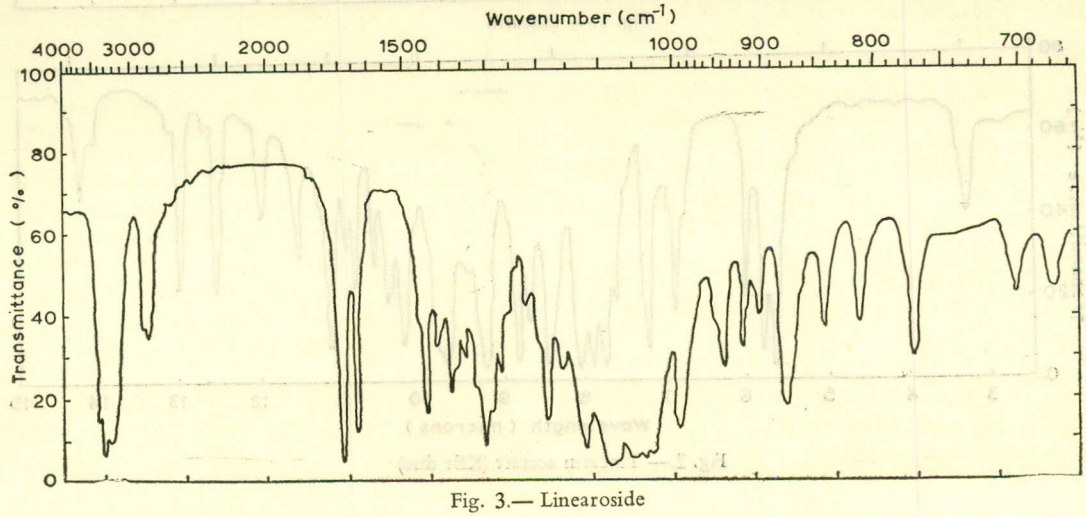

Fig. 3.— Linearoside

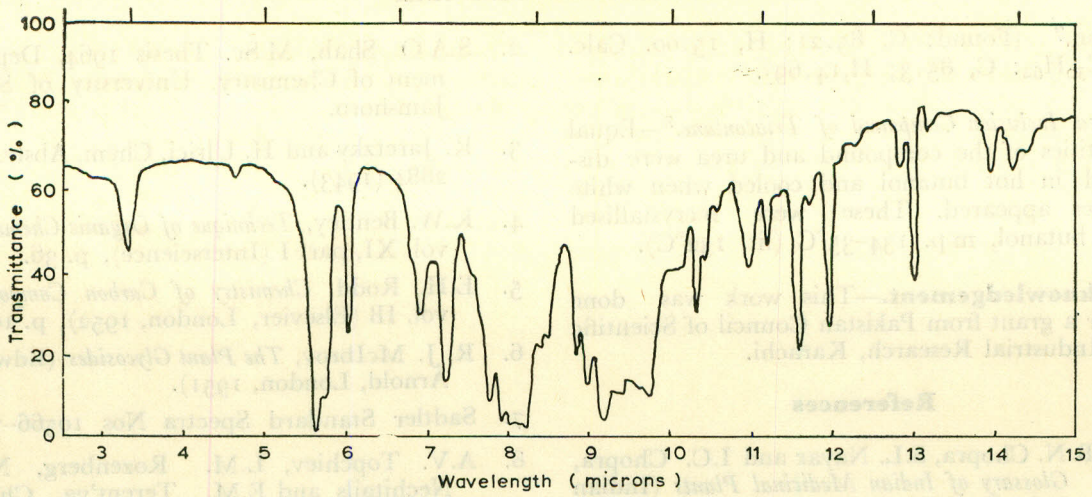

Fig. 4.— Linearoside acetate (KBr disc)

(25 ml) were shaken vigorously. More KOH solution was added to make the reaction mixture alkaline and it was again shaken when some solid settled at the bottom which was crystallised (0.08 g) from benzene–petroleum ether twice, m.p. 156–58°C. Found: C, 66.84; H, 5.12. $C_{45}H_{42}O_{14}$ requires: C, 67.00; H, 5.21%. Its IR spectrum showed bands at 3100, 2800, 1820, 1750, 1720, 1625, 1620, 1475, 1375, 1300, 1220, 1175, 1000, 935, 812, 710 680 cm^{-1} (Nujol mull).

Hydrolysis of Linearoside.—The compound (1 g) dissolved in 10% sodium hydroxide (10 ml), warmed on water bath for 1 hr, cooled, acidified with dilute hydrochloric acid and dried under vaccuum. The residue dissolved in alcohol to remove sodium chloride. The alcohol evaporated and the residue crystallised (0.2 g) from alcohol–acetone at room temperature, m.p. 195–200°C. Its IR gave peaks at 3340, 2810 1680, 1625, 1510, 1380, 1060, 940, 910, 870, 850, and 780c m^{-1} (KBr disc).

Heating of Linearoside above the Melting Point.—The compound (1 g) was heated at 250–80°C and the outcoming gas was indentified as carbon dioxide. The heating was continued till the evolution of carbon dioxide ceased. The residue was dissolved in alcohol, decolorised and dried. It crystallised (0.03 g) from benzene after 15 days at room temperature, m.p. 165–68°C. Its IR showed peaks at 3125, 2700, 1625, 1550, 1375, 1320, 1175, 1120, 1040, 960, 915, and 745 cm^{-1} (KBr pellet). The band of carbonyl group 1690 cm^{-1} in the original compound) disappeared.

Oxidation of Linearoside with CrO_3.—A solution of CrO_3 (6.0 g), and dilute H_2SO_4 (175 ml) was added slowly to linearoside (5.0 g). The reaction mixture was left at room temperature for 24 hr and then extracted with ether 8–10 times. The ether was recovered, and the residue crystallised from chloroform–petroleum ether (50–70°C) at room temperature. It was recrystallised from benzene to give a needle-like product, m.p. 125–26°C. The mixed m.ps of the product and its acetate were undepressed with those of linearin and its acetate. The IR spectra were superimposible.

Mannitol.—The dry alcoholic extract after the removal of linearoside was dissolved in alcohol, concentrated and cooled when needles of mannitol separated. They were recrystallised similarly when pure mannitol (30 g, 0.6%), m.p. 164–66°C, separated. The mixed m.p. with an authentic sample of mannitol was undepressed. The IR spectrum was superimposible on that of authentic mannitol.

After the removal of mannitol, glucose and mannose were identified by paper chromatography and as phenyl hydrazone derivatives, from the alcoholic solution.

Triacontane.—The plant (10 kg) after cold extraction with 90% alcohol was heated under reflux with benzene on water bath for 3 hr three times. The benzene solution was concentrated and cooled when crystalline product separated. The compound was purified by passing through a column of alumina (E. Merck) with benzene as the solvent. The eluates were concentrated and cooled when white, shining plates of triacontane (12 g, 0.12%, m.p. 66–67°C) separated.

Its IR spectrum (in KBr) shows absorption at 2950 2857, 1465, 730 and 718 cm^{-1} which is characteristic of an aliphatic saturated hydro-

carbon.[7] (Found: C, 85.21; H, 15.00. Calc. for $C_{30}H_{62}$: C, 85.3; H,14.69).

Urea Inclusion Compound of Triacontane.[8]—Equal quantities of the compound and urea were dissolved in hot butanol and cooled when white needles appeared. These were recrystallised from butanol, m.p. 134–35°C (lit. 132°C).

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