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CHEMICAL CONSTITUENTS OF CORCHORUS OLITORIUS AND CORCHORUS CAPSULARIS (JUTE)

Part II.*—Isolation of Corosin and β-Sitosterol from Roots

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A new compound, now designated as *corosin*, $C_{30}H_{46}O_7$, m.p. 292–293° (dec.)$[\alpha]_D^{26°}$ +39° (*c*, 0.5% in methanol) and β-sitosterol, m.p. 137–138°C, have been isolated from the roots of *Corchorus capsularis* and *Corchorus olitorius* plants. Corosin gave an acetate, m.p. 257–258°C, $C_{34}H_{50}O_9$, $[\alpha]_D^{25°}$ −4.5° (*c*, 0.96% in methanol). Corosin on refluxing in ethanol containing hydrochloric acid gave a product now designated as *corosic acid*, m.p. 285–286° (dec). $C_{30}H_{44}O_6$, $[\alpha]_D^{26°}$ +188° (*c*, 0.76% in methanol). On acetylation corosic acid gave an *acetate*, m.p. 247–249C, $C_{34}H_{48}O_8$, $[\alpha]_D^{26°}$ +127° (*c*, 0.9% in methanol).

Earlier chemical investigations on the jute plants were mainly carried out on the seeds and leaves of *Corchorus capsularis* plant.[1–5] Raffinose, jute oil, β-sitosterol, m.p. 137–8°C, β-sitosterol D-glucoside, m.p. 294–6° and strophanthidin, m.p. 175–6°C are the principal components reported so far. The identity and purity of corchoside A, m.p. 190–92°C and corchoside B, m.p. 137–40°C, have not yet been established.

A systematic examination of the jute plant has now been undertaken in these laboratories. No investigations have so far been reported on the roots of the plant. Any useful product which may be present in the roots, if isolated, will be of great significance to the cultivators of this cash crop. The present investigation was carried out on freshly collected roots from the harvested jute plants. The roots were extracted with ethanol at room temperature following the procedure of Manzoor-i-Khuda.[16–21] The ethanolic extractives were evaporated under reduced pressure to a mainly aqueous concentrate. The precipitated solid was collected after cooling and purified through fractional crystalisations (charcoaling). The less soluble fraction was first leached out with petroleum ether, then the residue purified by fractional precipitation (methanol–water) to give a new compound after repeated crystallisations from pure methanol, m.p. 292–293° dec. now designated as *corosin*,[18] $C_{30}H_{46}O_7$, $[\alpha]_D^{26°}$ + 39° (*c*, 0.5% in methanol). Corosin tends to be precipitated as a gel, but on very slow crystallisation from ethanol (or methanol) well-defined hexagonal plates were obtained. The more soluble fractions gave after further purification β-sitosterol, m.p. 137–38°C. On silica gel G thin layer chromatography, corosin gave an R_f value of 0.08 when developed with 30% ethyl acetate in benzene as compared to an R_f value of 0.7 for β-sitosterol. After a detailed investigation the most suitable solvent for thin layer chromatography on silica gel-G (250μ thick) plates was found to be chloroform–ethyl acetate–formic acid mixture (55:40:5) which gave an R_f value of 0.47 for corosin (60 min development). Corosin was obtained from both *C. olitorius* and *C. capsularis* roots and is soluble in methanol, moderately soluble in ethanol and tetrahydrofuran and sparingly soluble in other usual organic solvents. Corosin is acidic in nature and dissolves readily in dilute aqueous ammonia at room temperature. The aqueous mother liquors of the plant extracts from *C. olitorius* and *C. capsularis* both showed clearly the presence of raffinose, glucose, fructose and arabinose. Sucrose and galactose were detected in traces.[17] Additionally, some crystalline inorganic salts were obtained from the concentrated aqueous solution.

The $C_{30}H_{46}O_7$ compounds reported in the literature includes presenegenin isolated from *Polygala senega*, m.p. 310–11°, $[\alpha]_D$+91° (*c*, 1.16% MeOH), triacetate, m.p. 219–220°, $[\alpha]_D$+110° (*c* 2.32% $CHCl_3$)[19–20] which has been assigned the structure 2 β, 3 β, 27-trihydroxyl olean-2-ene-23,28-dioic acid. The three other hydroxy acids reported in the literature include lucernic acid $[\alpha]_D^{25°}$+12.4° (*c* 0.6 pyr.) (isolated as its acetate)[21] isolated from alfalfa, hydroxysenegenin m.p. 246–253°; $[\alpha]_D^{22}$+5.7°, derived from senegenin,[20] somalin m.p. 197–8°; $[\alpha]_D^{19}$+9.5°[22] and barringtonic acid[23] which appears to be same as compound D, m.p. 285°; $[\alpha]_D^{30}$+19.6°, from the wood of *Barringtonia acutangula*.[24] The structure assigned to hydroxysenegenin shows its primary hydroxyl group (C-27) attached to the carbon 12 instead of at 14 as in presenegenin and the double bond is at 13–14 position of the ring skeleton. Another triterpenic dicarboxylic acid $C_{30}H_{46}O_7$ reported from

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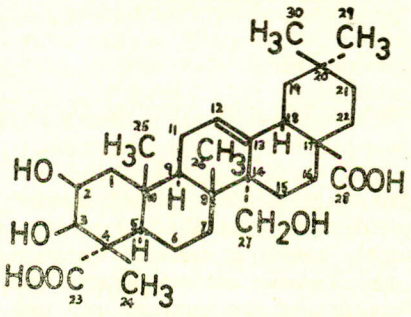
Barringtonia acutangula has been assigned the structure 2,3,19-trihydroxyoleane-12-ene-23,28-dioic acid. Other $C_{30}H_{46}O_7$ compounds reported, e.g. cucurbitacin D,[25] are not acidic in nature.



Presenegenin

Corosin, obtained as well-formed hexagonal plates, is a new product in the triterpenoid group of compounds. It gave a green colour in Salkowski test[27] and a pink colour in the Liebermann–Burchard[28,29] reaction. In reaction "A" of Whitby[30] the sulphuric acid layer gave a light greenish yellow colour, the chloroform layer gave a light blue colour changing to green; the original substance was only sparingly soluble in chloroform.

Corosin gave main peaks at 3570m, 3410m (broad), 2970s, 2925s, 2860s, 1685s (may be a compound peak), 1565w, 1460m, 1375m, 1263m, 1235ms, 1152m, 1070m, 1055m, 1040m, 970mw, 960mw, 940mw, 908w, 867w, 770w, and 645w cm^{-1} in its IR spectrum taken in KBr pellet (Fig. 1). Additionally, it showed absorptions in between 2700–2300 cm^{-1} indicating presence of acidic protons. Various inflexions (shoulders) were also noted in the IR spectrum e.g. at 2975s and 1655mw

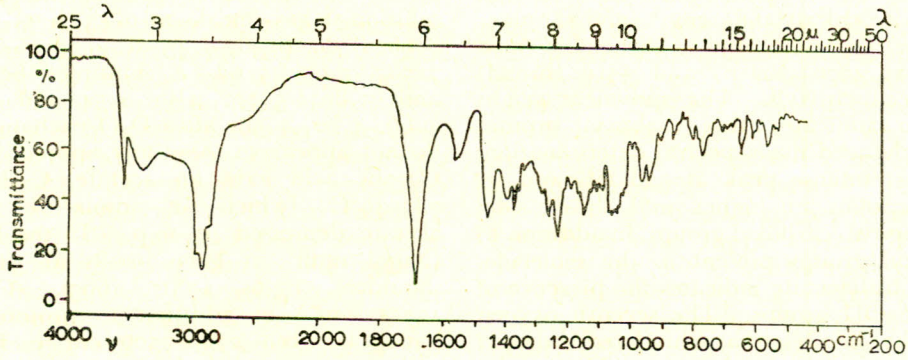


Fig. 1. — Infrared spectrum of corosin (KBr pallet)

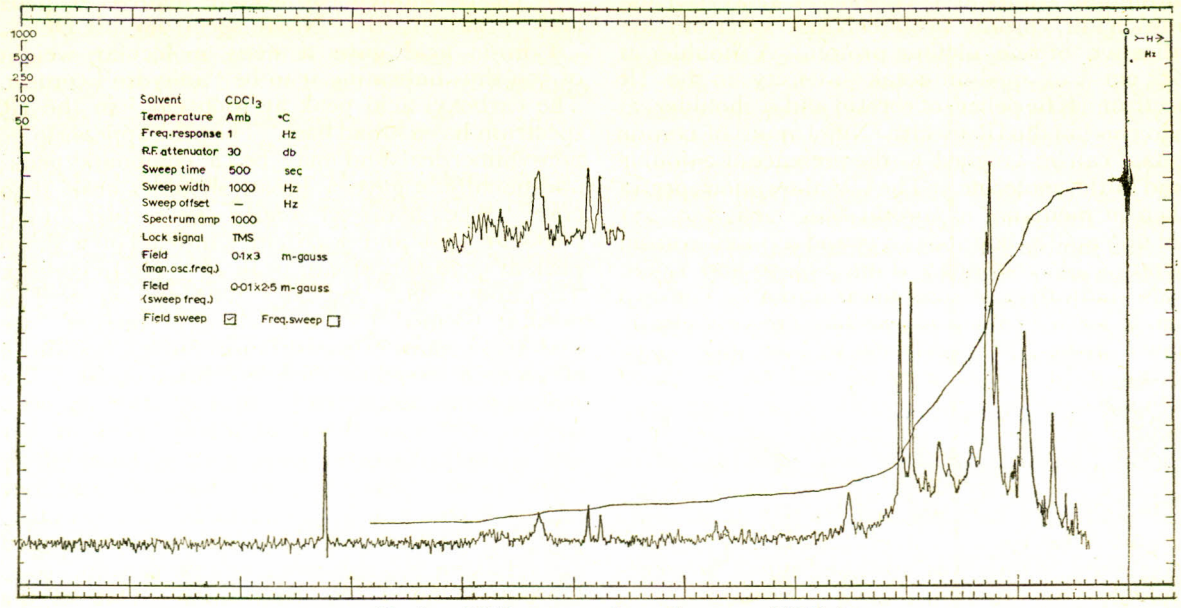


Fig. 2. — NMR spectra of corosin acetate ($CDCl_3$)

cm^{-1}. In the NMR spectrum taken in CD_3OD solution it gave peaks at 9.59, 9.43, 9.26, 9.18, 9.12, 8.03, 8.92, 8.89, 8.83, 8.73, 8.67, 8.62, 8.42, 8.33, 8.24 8.06, 8.03, 7.95, 7.78, 7.5, 7.25, 7.15, between 6.14 and 5.90 (multiplet), and triplet at 4.75 4.71 and 4.65τ. Corosin gave an acetate, m.p. 257–58°C, $C_{34}H_{50}O_9$, which gave peaks at 3500m, 1735s, 1725i, 1690s, 1645i, 1640i, 1380s cm^{-1} in the IR spectrum (Nujol mull) in addition to the acidic protons absorption (2700–2500 cm^{-1}). In the NMR spectrum corosin acetate (in $CDCl_3$ after D_2O exchange) showed bands at 9.52, 9.43, 9.32, 9.19, 9.10, 9.07, 8.96, 8.90, 8.82, 8.75, 8.73, 8.59, 8.38, 8.3, 8.2, 8.18, 8.05 (one acetate methyl), 7.94 (one acetate methyl), 7.52, 7.48, 7.44, 7.42, 5.24, 5.14, multiplet between 4.50 and 4.16 and triplet at 4.72, 4.68 and 4.64τ ($CDCl_3$ solution (Fig. 2).

From the spectral studies it can be seen that corosin gives a peak at 1685 cm^{-1} (and shoulder near 1700 cm^{-1}) possibly due to carboxylic acid groups and at 3570 (sharp) and 3410 (broad) assigned to free hydroxyls. The equivalent weight determination indicates two carboxyl groups. The D_2O exchanged mass spectral determinations gave the most intense peak at 520 followed by weaker absorptions at higher and lower side, thus indicating two carboxyl groups, in addition to other hydroxyl groups present in the molecule. Formation of a diacetate indicates the presence of two available OH groups. The seventh oxygen atom may either be present as a hindered hydroxyl group, an ether oxygen or a ketone. The triplet at 4.75, 4.71 and 4.65τ (CD_3OD solution) in corosin and triplet at 4.72, 4.68 at 4.64τ ($CDCl_3$ solution) in corosin acetate NMR spectrum are indicative of one olefinic proton. A shoulder at 1655 cm^{-1} of medium-weak intensity in the IR spectrum (KBr pellet) of corosin and a shoulder at 1650 cm^{-1} in the IR spectra (Nujol mull) of corosin acetate can be assigned to the presence of a double bond in the molecule. The UV absorption spectra taken in methanol of corosin λ_{max} 206, 235, 271 and 295 mμ (ε2,800; 625; 575 and 23) and corosin acetate λ_{max} 206 and 250 mμ (ε9,250 and 1,000) is also indicative of such unsaturation. The two acetate methyls of corosin acetate are very clearly shown in the NMR spectrum at 8.05 and 7.94τ. Corosin did not form an oxime and was recovered unchanged on refluxing in methanol with hydroxylamine hydrochloride in presence of pyridine. It seems most likely that the seventh oxygen atom is present as a hindered hydroxyl group in corosin.

On refluxing with ethanol containing a small amount of hydrochloric acid, corosin gave a reaction product which was separated into a benzene-insoluble fraction, which gave a crystalline substance, now designated as corosic acid, $C_{30}H_{44}O_6$ m.p. 285–6°C. Corosic acid showed main IR bands at 3400m (broad) 2940s, 2875s, 1695s (a compound peak), 1470m, 1450m, 1432m, 1385m, 1370m, 1250m, 1192m, 1135m, 1130m, 1058ms, 1040m, 1025, 998w, 982m, 962m, 805mw, 750w, 710w, 645w, 615w and 600w cm^{-1} in addition to the proton absorption between 2700–2300 cm^{-1} region. Additionally various inflexions and broad shoulders were also noted at 2980–2940 (broad shoulder), 1650 (broad), 1405m cm^{-1}. On careful slow crystallisation from methanol the benzene-inosoluble solid gave hexagonal crystals. On thin layer chromatography (silica gel G with chloroform–ethyl acetate–formic acid, 55:40:5, development) it gave an R_f value of 0.5 (60-min. development) similar to corosin but a mixture of corosin and corosic acid separated clearly on chromatography of the mixture into two distinct spots on TLC under identical conditions indicated above. In the NMR spectrum taken in CD_3OD solution it gave peaks at 9.43, 9.11, 9.06, 9.01, 8.96, 8.89, 8.58, 8.45, 8.39, 8.28, 8.09, 7.96, 7.78; 7.67, 7.07, a multiplet between 6.02 and 5.86; 5.11; 4.52, 4.54 and a triplet at 4.66, 4.62, 4.58τ after D_2O exchange the peaks do not appear to show any appreciable changes. Corosic acid gave an acetate, $C_{34}H_{46}O_8$, m.p. 247–49°C (which on admixture with corosin acetate depressed its m.p.). In the IR spectrum (Nujol mull), it gave bands at 3450m (broad shoulder), 1730s, 1751i, 1695i, 1685s, 1655i, 1375s, cm^{-1} in addition to proton absorption bands between 2700–2500 cm^{-1}. In the KBr pellet spectrum with a higher resolution the acetate gave peaks at 1745s, 1710s, 1695s (inflexion cm^{-1}).

It clearly showed two acetate methyls at 8.02 and 7.92τ in the NMR spectrum ($CDCl_3$).

Corosic acid gave a mass molecular weight of 500 thus indicating it to be "anhydro" corosin. The carboxyl acid peak at 1690 cm^{-1} in the IR spectrum has a broad base. The peak appears to be nonsymmetrical and may be a compound peak; additionally it gave a broad shoulder near 1650 cm^{-1}. The hydroxyl region gave two broad peaks at 3400 and 3250 cm^{-1} instead of a sharp peak at 3570 and broad peak at 3410 in corosin. The above data indicates that corosic acid is possibly formed by elimination of one of the hydroxyl groups of corosin on refluxing corosin in ethanol in presence of hydrochloric acid. The mass spectral determination of the D_2O exchanged corosic acid gave the most intense peak at 501 and a slightly weaker peak at 502, followed by other weaker peaks thus indicating at least one readily exchangeable proton in addition to other protons. Corosic acid is recovered unchanged on refluxing in ammonia or 2N caustic soda solution followed by acidification. One of the hydroxyl group of corosin appears to be eliminated resulting in a double bond within the

molecule thus giving corosic acid. The NMR spectrum gave a triplet at 4.66, 4.62, and 4.58 assigned to olefinic protons—the IR spectrum also gave shoulder near 1650 cm^{-1} indicating unsaturation. In the UV spectrum taken in methanol solution corosic acid gave peaks at 207, 230 and 272 mμ (ε 9,600; 4,500 and 1,100) and corosic acid acetate gave peaks at 207, 232 and 272 mμ (ε 8,700; 4,800 and 650). In addition to the multiplet between 6.02 and 5.86 τ in the NMR spectrum a sharp peak of moderate intensity was also obtained at 8.28τ.

The weak UV absorption of corosin and corosic acid and their acetates in the higher wavelengths are probably due to the carboxyl groups present in the molecule. The peaks of moderate intensity at 230 and 232 mμ in the corosic acid and corosic acid acetate appears to be due to the newly formed double bond in the molecule.

The mass spectral analysis of corosin is given in Fig. 3. Metastable ion peaks at 482, 340 and 185 could be detected. These are probably derived from the parent peaks 518 (M^+), 472 ($M-H_2O$ and CO) and 219. The daughter ions at 500, 401 and 201 respectively appear to be derived from the parent ions through the process of elimination of H_2O, C_5H_{11} (or C_4H_7O) and H_2O respectively. The molecular fragmentation pattern indicates that initial elimination of a molecule of water is followed by elimination of a molecule of carbon monoxide thus giving rise to the peak at 472 (M-46).

The molecular fragmentation pattern of corosic acid as observed from its mass spectral analysis (Fig. 4) also indicates initial degradation through elimination of a molecule of water (482) followed by elimination of a molecule of carbon monoxide thus giving a peak at 454 (M-46).

The mass peaks of comparable intensites can be observed at 246, 219, 201, 187, 173, 159, 131, 105, 95, 81, 69, 41 and 28 in both corosin and corosic acid. It is however interesting to note that the intense peak of corosic acid at 119 (C_9H_{11}) appears only as a medium intensity peak in its parent compound corosin, whereas the base peak of corosin which appears at 146 ($C_{11}H_{14}$) is altogether absent in corosic acid. The intense peaks at 29 (base peak; 20% more intense than peak at 119) and 31 (10% more intense than peak at 119) observed in corosic acid are present as peaks of nominal intensity in corosin. The base peak of corosic acid at 29 and the peak at 31 possibly indicates the abundance of $HC\equiv O^+$ and $CH_2=OH^+$ moiety in corosic acid. The

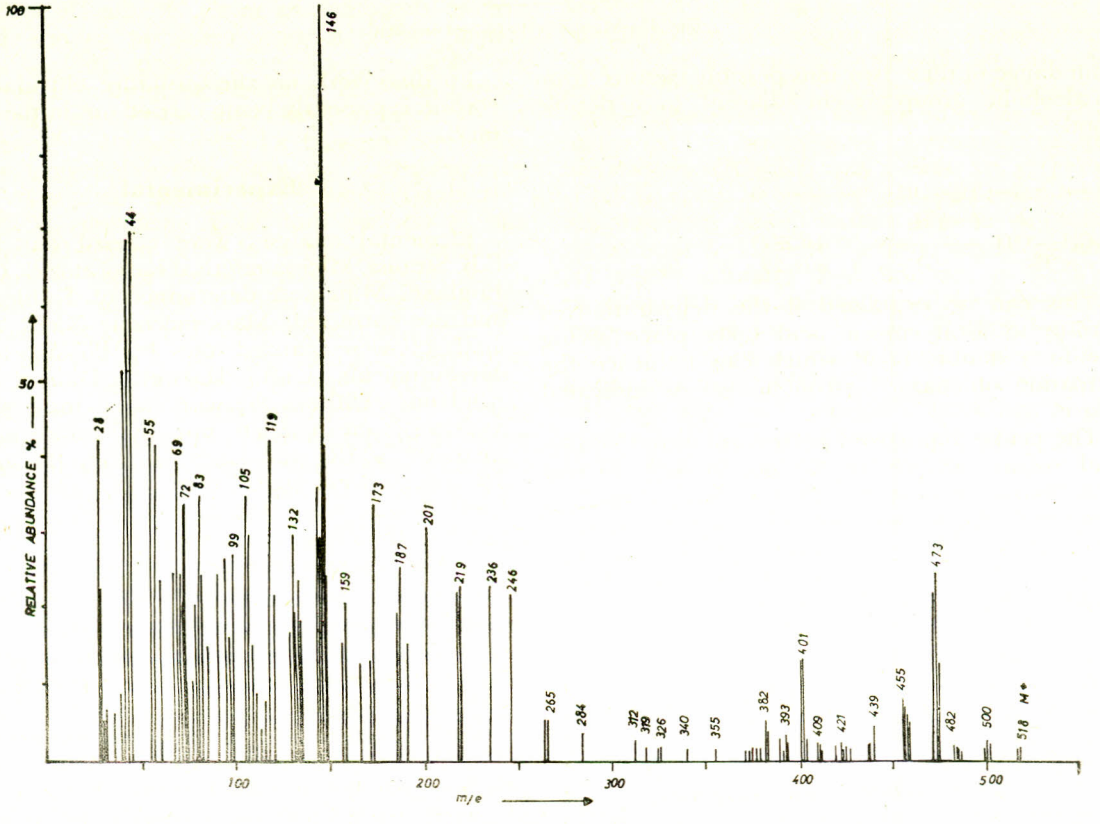


Fig. 3.—Mass spectra of corosin.

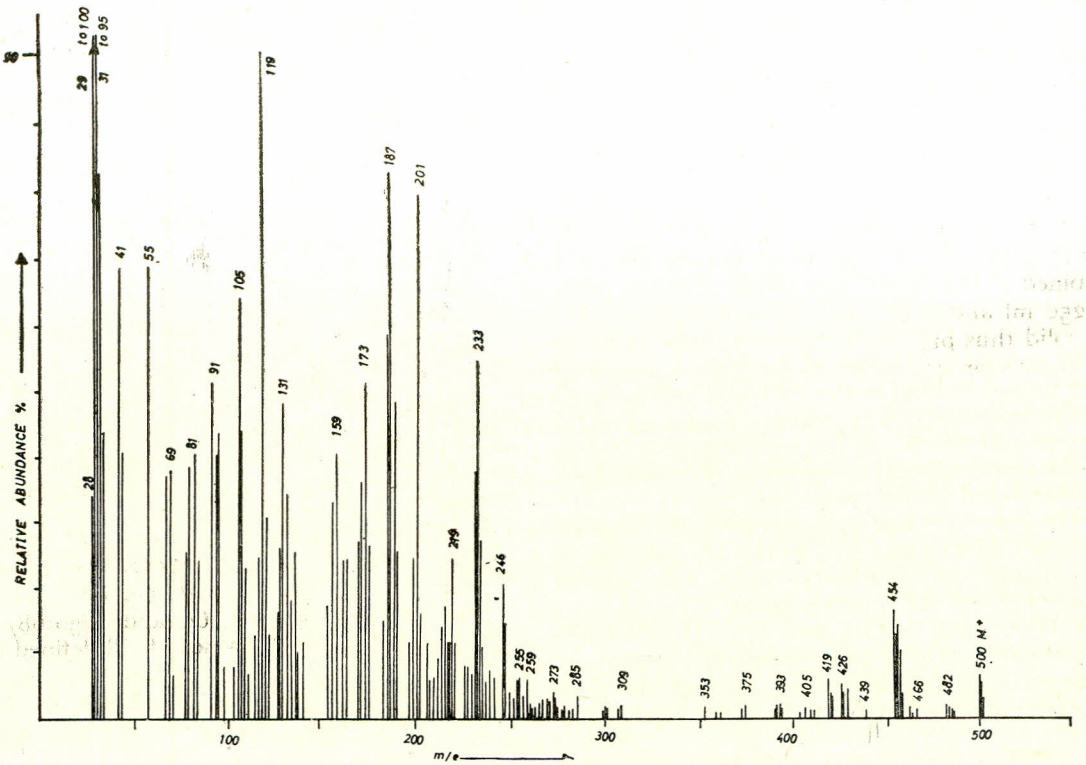
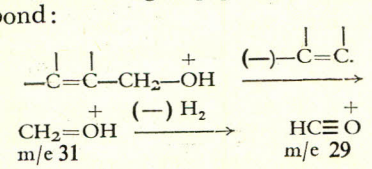


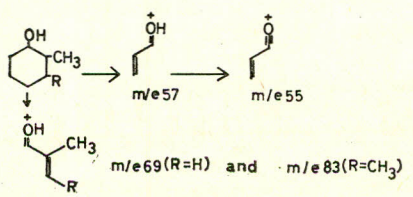
Fig. 4.—Mass spectra of corosic acid.

abundance of these two ions possibly results from an alcoholic group present adjacent to a double bond:



This can be explained if the dehydration of corosin to form corosic acid takes place giving rise to a double bond which can influence the formation of charged alcoholic ion as indicated above.

The peaks common to both corosin and corosic acid are at 28, 41, 43, 55, 69, 81, 95 and 105. The mass ions at 55, 57 and 69 are possibly derived from various alcoholic group or groups attached to differently substituted ring or rings in the molecules. The substituted alcohol will give rise to mass ions as follows:



Further work on the structure elucidation of corosin is presently being carried out in the laboratory.

Experimental

Elemental analyses were carried out by Dr. F.B. Strauss, Microanalytical Laboratories, Oxford, England, M.ps were determined on Köffler block and are corrected. Mass spectral, NMR and IR analyses were carried out by Physicochemical Measurements Unit, Harwell, Didcot, Berks., England. Unless otherwise stated mass spectral analyses were carried out by probe injection method, NMR was taken in CD_3OD solution, IR spectra were recorded in KBr pellet, and UV spectra was carried out in methanol solution. Some of the IR spectra were recorded with Perkin Elmer. 237E instrument and the UV spectra were recorded at the PCSIR laboratories, Karachi.

Extraction of Roots.—Fresh roots of the *Corchorus capsularis* (17 kg, dry wt 2.81 kg; collected from Savar area, near Dacca), were chopped into small pieces and soaked in ethanol (19.5 l) for 30 days. The alcohol was removed and the extraction repeated a second (10 days) and a third time (15 days). The extracts were separately concentrated *in vacuo* to 1000, 500 and 250 ml, respectively, and allowed to cool in a refrigerator.

The precipitates of first, second and third extracts were collected (Fraction A) and the aqueous mother liquors combined (Fraction B) and investigated separately.

Fresh roots of *C. olitorius* (4.2 kg, dry wt 1.37 kg, collected from Nayarhat area, Dacca) were chopped into small pieces and soaked in ethanol (9.8 l) for 9 days followed by second and third extractions for 18 and 17 days, respectively. The combined extracts were concentrated *in vacuo* to 1250 ml and allowed to cool in a refrigerator. The solid thus precipitated (Fraction A_1) and the mother liquor (Fraction B_1) were investigated separately, following the same procedure as for *C. capsularis* substances.

Isolation of Corosin.—The crude residue (Fraction A or A_1) was dissolved in methanol and repeatedly charcoaled till the solution was colourless. The solution was concentrated to some extent and allowed to cool in the refrigerator. A voluminous white precipitate of crude β-sitosterol was collected and purified further (Fraction C).

The mother liquor from the above crystallisation was charcoaled and concentrated and allowed to stand at room temperature (overnight). The precipitate was collected and after drying was leached out with petroleum ether (60–80°C). The insoluble residue was then repeatedly crystallised (charcoaled) from methanol, finally giving a microcrystalline powder, which on very slow crystallisation from ethanol (at room temperature) gave a crystalline (hexagonal plates) compound, now designated as *corosin*, m.p. 292–293° (dec.), $[\alpha]_D^{26°}+39°$ (*c*, 0.5% methanol).

The sample was dried at 50°C *in vacuo*. (Found: C, 68.69, 68.85; H, 9.1, 8.89; C-Me, 5.50%; equiv wt, 244; and mol wt (mass), 518.3238. $C_{30}H_{46}O_7$. 0.5 CH_3OH requires: C, 68.50; H, 9.05%; equiv wt (for 2 —COOH), 259; and mol wt (without methanol of crystalisation), 518.3244.)

C. Capsularis yielded about 0.2% (dry wt) corosin and *C. olitorius* gave about 0.08% corosin (dry wt). Corosin gave λ_{max} 206, 235, 271 and 295 mμ (ε 2,800, 625, 525 and 23) in the UV absorption spectra. To obtain well-defined crystals of corosin, it should be dissolved in dry ethanol by prolonged heating on water bath (*c* 0.5% solution). The solution should be allowed to crystalise slowly at room temperature through very slow evaporation over a period of month or more.

Corosin when dissolved in acetic acid containing some acetic anhydride and a few drops of concentrated sulphuric acid added to it, developed a pink colour (Liebermann–Burchard test).[28,29] The colour test when performed in chloroform solution (sparingly soluble) with a little concentrated sulphuric acid added (Salkolwaski test)[29] gave a faint green colour to the sulphuric acid layer. A small quantity of the substance was taken in chloroform and shaken with an equal volume of a reagent (50 vols concd H_2SO_4 and one vol Formalin), when the sulphuric acid layer gave a greenish-yellow colour, the chloroform layer (top) gave a light blue colour changing to green (reaction "A" of Whitby).[30]

Thin Layer Chromatography of Corosin.—A number of different mixtures were tried for corosin to determine the most suitable solvent for the thin layer chromatography of this substance. Silica gel-G plates (375 μ thick) were used for the trial runs. Various solvents e.g. ethanol–ethyl acetate–benzene (10:40:50 and 0:60:40); benzene–methanol–formamide (90:5:5); ammonia– ethylacetate–methanol (2:88:10); ethyl acetate–methanol–benzene (10:40:50); chloroform (equilibriated with 1N NH_4OH)–methanol (97:3) and methanol–5N ammonium hydroxide (80:20) were found unsuitable. Formamide–methanol–benzene mixtures (0.5:30:69.5; 1:30:69 and 2:30:68) were also unsatisfactory because of ill-defined spots resulting from the elution (R_f value 0.39 with 1:30:69 mixture). The plate thickness for subsequent experiments was kept at 250μ. Chloroform–methanol–formamide mixture (89:9:2; 80:18:2 and 70:28:2) were acceptable. The spots were, however, triangular in shape. The R_f values were 0.17 and 0.59 with 89:9:2 and 80:18:2 mixtures respectively. The best solvents were mixtures of chloroform–ethyl acetate–formic acid. The R_f values obtained were 0.467 and 0.354 with 55:40:5 and 65:30:5 of the solvent mixtures respectively.

Acetate of Corosin.—Corosin (0.1 g; m.p. 290–292° dec) was dissolved in dry pyridine (1.0 ml) and acetic anhydride (4.0 ml) and the mixture was allowed to stand at room temperature (overnight). The reaction product was evaporated under reduced pressure after dilution with benzene and dried to constant weight. The dry residue (0.11 g) was dissolved in ethanol, charcoaled and crystallised from methanol diluted with few drops of water (room temperature), to give crystals (0.73 g collected in three crops). On crystalisation from moist ethanol it gave *corosin acetate*, m.p. 257–258°C (rods), $[\alpha]_D^{26}$—4.5° (*c*, 0.96% in methanol). The sample was dried at 50°C in *vacuo*. (Found: C, 65.85, 66.02; H, 8.25, 8.30; O-Acet., 14.09%; equiv wt, 327; mol wt (mass) 602.3351. $C_{34}H_{50}O_9$.-H_2O requires: C. 65.78; H; 8.44; O-Acetate for two, 16.83%; equiv wt (for 2 —COOH) 310; mol wt (without water of crystallisation) 602.3396.) The sample was dried at 110°C *in vacuo*. (Found C, 67.96; H, 8.32%. $C_{34}H_{50}O_9$ requires: C, 67.75; H, 8.35%.) The R_f value was found to be 0.68 with 65:30:5 mixture of chloroform–ethyl acetate–formic acid. It had λ_{max} 206 and

250 mμ (ε 9,250 and 1000) in the UV absorption spectra.

Corosic Acid.—Corosin (0.6 g, m.p. 290–292°C dec.) was dissolved in absolute ethanol (150 ml) and refluxed with concd HCl (2.1 ml) for 12 hr under N_2 atmosphere. The reacted mixture was evaporated under reduced pressure and the distillate was collected separately in ice–salt bath. The residue was mixed with benzene and absolute ethanol and dried to constant weight 0.554 g. The volatile fraction collected at the trap was petroleum ether soluble and was mixed with the benzene-soluble fraction of the reaction product (0.205 g).

The dry residue was leached with benzene and the benzene-insoluble portion (0.344 g) was dissolved in ethanol, charcoaled and crystallised from pure ethanol at room temperature to give corosic acid, m.p. 285–286°C (dec) (0.19 g; hexagonal crystals; rhombic elongated crystals from methanol), $[\alpha]_D^{26°}+188°$ (*c*, 0.76% in methanol).

Analysis.—Sample dried at 50°C *in vacuo*, (Found: C, 70.82, 70.60; H, 8.93, 8.80%, equivalent wt 302; mol wt (Rast), 595; mol wt (mass), 500.1331. $C_{30}H_{44}O_6$. $0.5CH_3OH$ requires: C, 70.80; H, 8.97%, equivt wt (for 2—COOH), 258; mol wt 500.3138. Found: sample dried at 100°C *in vacuo*: C, 72.40; H, 9.11%. $C_{30}H_{44}O_6$ requires: C, 72.0; H, 8.9%.

The best solvents for TLC separation were chloroform–ethyl acetate–formic acid mixture, whence R_f value of 0.5 and 0.379 were obtained with 55:40:5 and 65:30:5 mixture respectively: with these solvent systems corosin and corosic acid could be clearly separated from their mixtures. It had λ_{max} 207, 230 and 272 mμ (ε 9,600, 4,500 and 1,100) in the UV absorption spectra.

Acetate of Corosic acid.—Corosic acid (0.08 g m.p. 284–286°C (dec) was dissolved in a mixture of pyridine (0.75 ml) and acetic anhydride (3 ml), and allowed to stand at room temperature (overnight). The mixture was then evaporated under reduced pressure with slight addition of benzene and dried to constant weight (0.10 g). The residue was dissolved in methanol, charcoaled and crystallised from dilute methanol to give corosic acid acetate, m.p. 247–249°C (palm-leaf-like cluster of needless) $[\alpha]_D^{26°}$ + 127° (*c*, 0.98% methanol).

Analysis.—Sample dried at 110°C *in vacuo*, Found: C,69.45; H, 8.49; O-Acetate, 18.51%. $C_{34}H_{48}O_8$ requires: C, 69.83; H, 8.27; O-Acetate, (for 2) 14.18%. The R_f value was found to be 0.65 with chloroform-ethyl acetate–formic Acid (65:30:5). It had λ_{max} 207, 323 and 272 mμ (ε 8,700, 4,800 and 650) in the UV absorption spectra.

Alkali Treatment of Corosic Acid.—Corosic acid (10 mg) was dissolved in an aqueous solution (2 ml) containing ammonia solution (*d*, 0.90; 1.0 ml) and kept at room temperature overnight. It was heated on steam bath and concentrated under reduced pressure. The acidified (HCl) solution was filtered and made free from HCl. The solid was dried *in vacuo* and on TLC gave an identical spot as corosic acid.

The uncoverted corosic acid (9.5 mg) obtained from the above reaction was dissolved in 2N caustic soda (3 ml) and left at room temperature. It was refluxed for 75 min, cooled and acidified (HCl). It was filtered and made free from acid and dried *in vacuo*. On TLC it gave an unchanged spot as corosic acid and also separated into two spots on TLC of a mixture with corosin. On crystallisation from ethanol it gave unchanged corosic acid, m.p. 285–86°C dec.

Hydroxylamine Hydrochloride Treatment of Corosin.—Corosin (10 mg) was dissolved in dry methanol (20 ml) and hydroxylamine hydrochloride (10 mg) was added to it. Two drops of dry pyridine was added to the mixture and was allowed to reflux for 1 hr. It was kept overnight at room temperature and evaporated to dryness under reduced pressure. The solid residue was triturated with distilled water, filtered and dried (8.0 mg). The powdery solid on TLC gave an identical spot as corosin. On crystalisation from ethanol it gave unchanged corosin m.p. 290–92°C dec.

Isolation of β-Sitosterol.—The crude solid (fraction C) from the alcoholic extracts of roots (1st crop from fractions A or A_1 crystallisations) was dissolved in methanol and repeatedly charcoaled until a colourless solution was produced. The solution was allowed to cool in a refrigerator and the voluminous precipitate formed was purified by repeated crystallisation from petroleum ether to give β-sitosterol, m.p. 137–138°C, $[\alpha]_D^{26°}$—32° (*c*, 1.0% in $CHCl_3$) and mixed m.p. with authentic specimen was undepressed.

Analyses.—Found: sample dried at 50°C *in vacuo*: C, 82.52; H, 11.87%. $C_{29}H_{50}O$,$0.5H_2O$ requires: C, 82.2; H, 12%. Found: sample dried at 110°C *in vacuo*: C, 83.9; H. 12.23%. $C_{29}H_{50}O$ requires: C, 83.98; H, 12.54%.

Thin layer chromatography on silica gel G, with 50% ethyl acetate in benzene elution, showed it to be a uniform product with an R_f value of 0.85.

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