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Investigation of the Non-basic Constituents of *Lythrum anceps* MAKINO

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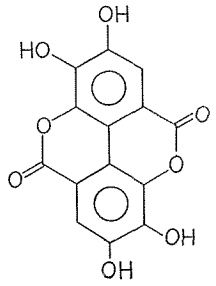
Investigation of the non-basic constituents of *Lythrum anceps* MAKINO led to isolation and characterization of ellagic acid and betulic acid.

We have investigated the alkaloidal constituents of *Lythrum anceps* MAKINO and elucidated the structure and absolute configuration of the novel fourteen alkaloids isolated so far.[1] Now, we carried out the investigation for the non-basic constituents of the same plant source.

The whole ground part of the flowering plant was extracted with methanol and the extract was separated into basic and non-basic fractions. The crude non-basic part was treated with methylene chloride to leave an insoluble solid, which was crystallized and purified as yellow needles, m.p. $>360°$. This compound was found to have the molecular formula $C_{14}H_6O_8$, on the basis of analysis and mass spectral data. Its UV and IR spectra suggested the presence of aromatic ring(s), ester group(s), and phenolic hydroxyl group(s) in the molecule. On methylation with diazomethane it gave a crystalline tetramethyl ether, while it, on acetylation with acetic anhydride and pyridine, afforded a crystalline tetra O-acetate. Consideration of these facts and site of unsaturation led to an assumption that it might be ellagic acid (**1**).[2] Hence, we synthesized ellagic acid and compared it with the natural product. Consequently, their complete identity was firmly established.

The methylene chloride soluble fraction was chromatographed on silica gel column to give betulic acid by elution with methylene chloride. Subsequent elution with acetone(10%)-methylene chloride gave a mixture of oleanolic and ursolic acids. The presence of β-sitosterol and diisobutyl phthalate in the root was also recognized.



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EXPERIMENTAL

Ellagic acid. The dried plant was extracted with methanol under reflux. The extract was concentrated to give a muddy residue, which was suspended with 0.5% hydrochloric acid and allowed to stand under occasional stirring. Filtration effected the separation of alkaloid from non-alkaloidal portion. The residue (non-alkaloid portion) was dried and extracted with methylene chloride. The methylene chloride soluble fraction will be described in the Betulic acid section. The solid which was insoluble in methylene chloride was dissolved in methanol and the solution was refluxed with charcoal. Usual treatment of the filtrate separated from charcoal gave a crystalline compound, which was recrystallized from methanol to yield a pure compound as yellow needles, m.p. $>360°$ (Yield: 1.8% of the dried plant). *Anal.* Calcd. for $C_{14}H_6O_8$: C, 55.64; H, 2.00; M.W. 302. Found: C, 55.35; H, 2.09; M^+ m/e 302. IR $\nu_{max.}^{KBr}$ cm^{-1}: 3550 (OH), 1700, 1200 (ester), 1620, 1580 (aromatic ring). UV λ_{max}^{MeOH} nm 255 (ε 40800), 366 (ε 8800). On addition of alkali, the absorption shifted to the longer wave-length. Its identity with the synthesized ellagic acid was firmly established (m.p., IR, UV). Its methylation with diazomethane gave a tetramethyl ether, m.p. $>300°$. *Anal.* Calcd. for $C_{18}H_{14}O_8$: M.W. 358. Found: M^+ m/e 358. A usual acetylation with acetic anhydride and pyridine afforded a tetraacetate, m.p. $>300°$. *Anal.* Calcd. for $C_{22}H_{14}O_{12}$: M.W. 470. Found: M^+ m/e 470.

Synthesis of ellagic acid. Gallic acid (5 g) was dissolved in acetic acid (50 ml) and the solution was heated under reflux, to which sulfuric acid (2.5 ml) was added. Subsequently, finely powdered potassium persulfate (5 g) was gradually added to the mixture. A somewhat violent reaction occurred and the clear solution rapidly changed to brown. The reaction mixture was allowed to stand for 30 min, after the reaction got gentle. Then, the mixture was poured into water and the precipitate was collected. After its treatment with charcoal in methanol at reflux and filtration, methanol was evaporated off to leave a crystalline residue, which was recrystallized from methanol to yield yellow needles (1.8 g), m.p. $>360°$. *Anal.* Calcd. for $C_{14}H_6O_8$: C, 55.64; H, 2.00. Found: C, 55.42; H, 2.06.

Betulic acid. The foregoing methylene chloride soluble fraction was chromatographed on silica gel column. The methylene chloride eluent gave betulic acid as colorless needles, m.p. 283–285° (yield 1.4 g from 50 g of the methylene chloride soluble substance). Liebermann-Burchard test: positive. *Anal.* Calcd. for $C_{30}H_{48}O_3$: M.W. 456. Found: M^+ m/e 456. IR $\nu_{max.}^{KBr}$ cm^{-1}: 3460, 1690, 1645, 890. NMR δ_{ppm} ($CDCl_3$): 0.7~1.3 ($6\times CH_3$), 3.45 (1H, t, J=8 Hz), 4.64, 4.73 (each 1H, s, $=CH_2$). Its methylation with diazomethane gave methyl betulate as colorless needles, m.p. 225–227°. *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 78.85; H, 10.68. The comparison with an authentic sample of methyl betulate confirmed their identity (m.p., m.m.p., IR and NMR spectra).

Oleanolic acid and Ursolic acid. The acetone (10%)-methylene chloride eluent of the foregoing chromatography gave colorless needles, m.p. 232–236° (yield 0.5 g from 50 g of the methylene chloride soluble substance). Liebermann-Burchard test:

positive. *Anal.* Calcd. for $C_{30}H_{48}O_3$: M.W. 456. Found: M^+ m/e 456. IR $\nu_{max.}^{KBr}$ cm^{-1} 3400, 1690. NMR δ_{ppm} ($CDCl_3$): 0.7~1.7 ($7\times CH_3$), 3.5 (1H, t, J=8 Hz), 5.5 (1H, s). The gas chromatography of the methylated product (CH_2N_2) showed it to be a mixture of methyl oleanolate and methyl ursolate (1:1) by comparison of the retention time with the authentic samples.

β-Sitosterol. From the non-basic fraction of the root, β-sitosterol was isolated as colorless needles, m.p. 137.5–139.5° by a column chromatography on alumina. The acetate, m.p. 122–124°. *Anal.* Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48. Found C, 81.54; H, 11.44. Its comparison with the authentic sample of β-sitosterol acetate confirmed their identity (m.p., m.m.p., and IR spectrum).

Diisobutyl phthalate. From the benzene eluent of the alkaloid fraction of the root, an oily neutral substance was isolated. IR $\nu_{max.}^{KBr}$ cm^{-1}: 1725, 1608, 1580, 1290. NMR δ_{ppm} ($CDCl_3$) 0.98 (12H, d, J=6 Hz), 2.0 (2H, m), 4.1 (4H, d, J=6 Hz), 7.5 (4H, m). Its hydrolysis with 10% KOH-MeOH under reflux for 5 hrs. gave phthalic acid. The retention time of its gas chromatogram was completely identical with that of the authentic sample of diisobutyl phthalate.

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