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# Isolation of Solanesol from Korean Native Tobacco

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## 한국산 재래종 잎담배중의 Solanesol의 분리

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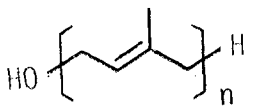
Solanesol has been isolated from aged Korean native tobacco leaf(*Hyangcho* and *Sohyang*) by water pretreatment, hexane extraction and column chromatography on Florisil in yields of 0.41% and 0.31% dry wt., respectively. Thin-layer densitometric analysis of the hexane extracts of samples(*Hyangcho Sohyang*) has shown that the amounts of solanesol present are 0.74% and 0.52% dry wt., respectively.

Solanesol(3, 7, 11, 15, 19, 23, 27, 31, 35-nonamethyl-2, 6, 10, 14, 18, 22, 26, 30, 34-hexatriacontanonaen -1 -ol) is an isoprenoid alcohol which was first isolated from tobacco in 1956[1]. It was originally ascribed the decaprenoid structure (1;n=10), but recent work indicates the nonaisoprenoid structure(1;n=9)[2~4]



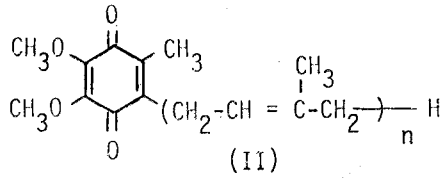
(I)

Solanesol and its derivatives, such as solanesyl esters, account for 1.9%~2.5% of the aged cured leaves of different tobacco types[5]; hence solanesol is the most abundant terpenoid in tobacco.

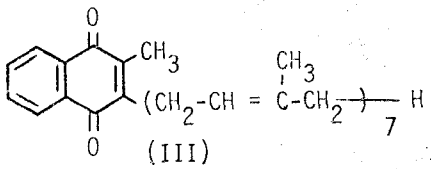
The physiological function of solanesol in green plants, remains undefined; however, in cured tobacco this compound together with other terpenes contribute to leaf aroma and smoke flavour[6].

Solanesol is main precursor of polynuclear aromatic hydrocarbons in cigarette smoke[7~10]. There does not appear to be a direct relationship between solanesol and leaf quality; however, it may be a contributor to diterpene in smoke and influence smoke aroma indirectly[11].

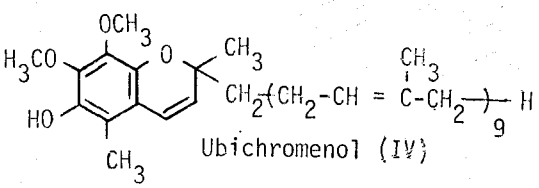
On the other hand, solanesol is a very important compound because of its possible use in



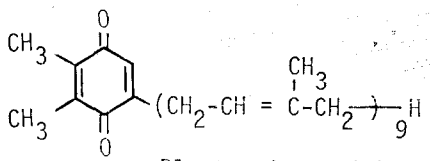
(II)



(III)



Ubichromenol (IV)



Plant quinone (V)

the preparation of drugs such as coenzyme $Q_n$ ($Q_7$, $Q_8$, $Q_9$, $Q_{10}$) (**II**), vitamin $K_2$(**III**) and related compounds(**IV, V**).

This paper describes isolation of total solanesol from Korean native tobacco(*Hyangcho* and *Sohyang*) by column chromatography.

## Experimental

Melting points were determined with Fisher-Johns apparatus, and were uncorrected. $^1H$ N M R spectrum was recorded on a Varian EM-360 spectrometer in carbon tetrachloride with TMS as an internal standard, ir spectrum with Perkin Elmer 337. The TLC plates were made by mixing silica gel G type 60(Merck) 35g, with chloroform: methanol(2:1, v/v) 100ml, and their chromatograms were developed with hexane: ethyl acetate(9:1, v/v) and detected by iodine vapor and phosphomolybdic acid. Densitometry was carried out using a Shimadzu C. S. 910.

The following tobacco samples were analyzed: Hyangcho and Sohyang(1978)-sun-cured, redried, aged tobacco leaf.

### Extraction and Isolation of Solanesol

The above mentioned samples, devoid of midrib, were equilibrated at laboratory conditions for two days and then ground in a Wiley Mill to pass through 30 mesh screen Water (10-15 times) was added. The mixture was stirred well for 3hr. at room temperature and was filtered. The water-extracted tobacco was air-dried below 50°C for several days, and cooled. To this is added *n*-hexane(10 times) and refluxed under a stream of nitrogen for 3hr. The mixture was filtered under suction and the procedure was repeated with residue. The combined extracts were hydrolyzed with 2N ethanolic potassium hydroxide under a stream of nitrogen. The reaction mixture was neutralized with 2N hydrochloric acid. Ethanol was evaporated under suction. The residue was extracted 3 times with *n*-hexane. The combined hexane extracts were washed with water and dried over anhydrous sodium sulfate. The dried extracts were concentrated to dryness below 40°C in vacuo. The residue was chromatographed on Florisil(60-100mesh, Sigma). The concentrated benzene/hexane eluate containing solanesol was subjected twice to preparative TLC on silica gel G(2mm) plates in hexane/ethyl actate(9:1). The silica gel G plates were sprayed with phosphomolybdic acid to develop with characteristic blue color; $R_f$ in hexane: ethyl acetate(9:1), 0.24. The concentrated benzene/hexane eluate containing solanesol was recrystallized with acetone at -27°C. M.P. 35-36°C. IR spectrum. νmax. ($cm^{-1}$): 3360(m), 2950-2875(s), 1670(w), 1450 (m) 1390(m), 1000(m), NMR spectrum($CCl_4$) δ: 1.58, 1.98, 2.75(broad s) 4.02, 5.1.

### Densitometry

Tobacco extracts Cca. 500ug) were chromatographed alongside solanesol standards on silica gel G.(2mm, Merck) in hexane: ethyl acetate (9:1) followed by treatment with phosphomolybdic acid spray reagent. Densitometry was carried out using a shimadzu C.S. 910 densitometer with a TLC attachment operating in the reflectance mode. The solanesol content of the samples was calculated from its calibration curve obtained for standards(peak area VS amount of solanesol).

## Results and Discussion

Water pretreatment prior to hexane extraction has several advantages. It leaches out approximately 50% of leaf components, reducing the tobacco load by half and thereby cutting down the solvent requirement by half for efficient extraction. The procedure described above is

**Table I.** Results of water extraction of tobacco.

| | Hyangcho | | Sohyang | |
|---|---|---|---|---|
| | O.T. | W.E.T. | O.T. | W.E.T. |
| Weight(g) | 1,000 | 520 | 1,000 | 540 |
| Solanesol(%)(Column chromatograpy) | 0.43 | 0.79 | 0.32 | 0.57 |
| Solanesol content(g)(Column Chromatograpy) | 4.30 | 4.11 | 3.20 | 3.09 |
| Loss due to water extraction(%) | — | 4.42 | — | 3.44 |
| Nicotine(%) | 5.52 | 1.90 | 2.08 | 0.47 |
| Nicotine content(g) | 55.2 | 9.88 | 20.8 | 2.55 |
| Loss due to water extraction(%) | — | 82.1 | — | 87.7 |

O.T.=Original tobacco
W.E.T.=Water extracted tobacco

**Table II.** Revovery of solanesol by column chromatography.

| Sample | *Hyangcho* (Water pretreatment) | *Sohyang* (Water pretreatment) |
|---|---|---|
| Solanesol(%)(Thin-layer densitometric analysis) | 0.74 | 0.52 |
| Isolated solanesol(%)(Column chromatography) | 0.41 | 0.31 |
| Solanesol recovery(%)(Column chromatography) | 55.4 | 59.6 |

important to carry out the experiments economically and conveniently. The loss of solanesol was negligible by the water pretreatment above (Table 1).

The recovery of solanesol from Hyangcho and Sohyang by column chromatography is shown in Table II.

Infrared and NMR spectrum of isolated solanesol were in agreement with those already published[12,13]. Melting point of isolated solanesol was different from that of Rowland and Giles[14] (mp 38°C and 42°C), but was identical with that of woolen[15].

The residue left after hexane extraction is suitable for making tobacco sheet. It has an extremely low nicotine content and is practically free from solanesol, the most important lipid component, known as a carcinogenic precursor from pyrolysis studies[16].

## Summary

1. The amounts of solanesol present in *Hyangcho* and *Sohyang* by thinlayer densitometric analysis are 0.74% and 0.52% dry weight respectively.
2. The yields of solanesol isolated from aged *Hyangcho* and *Sohyang* by water pretreatment, hexane extraction and column chromatography on Florisil are 0.41% and 0.31% dry weight respectively. Thus solanesol recovery is about 60%.
3. The column chromatography on Florisil is a suitable tool for isolation of solanesol from tobacco in laboratory, though it gives not so good yield and is a little tedious.
4. The residue left after hexane extraction is suitabe for making tobacco sheet.

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