Clovamides; L-Dopa Conjugated with trans- and cis-Caffeic Acids in Red Clover (Trifolium pratense)

Teruhiko YOSHIHARA, Hiroshi YOSHIKAWA, Sadao SAKAMURA and Tsutomu SAKUMA*

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo, Japan
*Hokkaido National Agricultural Experiment Station, Sapporo, Japan

Received February 20, 1974

The polyphenol compounds have been shown to be correlated with a resistance to the plant disease. Sakuma1) found that in red clover (Trifolium pratense) the quantity of the polyphenols increased during the process of the disease and the spore germination of Kabatiella caulivora was inhibited completely by the active peroxidase-polyphenols system in the clover extract.

Therefore, attempts to isolate the polyphenols from the red clover led to confirmation of novel polyphenols named trans- and cis-clovamide (I and II) and known phaselic acid2) (III).

Fresh leaves and stems of the plants (9.3 kg) were chipped and boiled in water. The filtrates were treated with neutral lead acetate and the resulting precipitates were treated with hydrogen sulfide. The filtrates were concentrated and extracted with ethyl acetate. The extract was concentrated in vacuo and fractionated by the method of Sondheimer.3) Fractions collected were monitored by UV absorption at 280 nm and separated mainly into two fractions (F1 and F2).

The F1-compound (III) was obtained as a pure oily substance, 6015 mg (0.065% yield), [α]_D^21 +21.2° (c=4.2, H2O), PC; Rf 0.63 in 2% acetic acid. The IR spectrum revealed absorption band at 1700~1720 cm⁻¹ (−C=O−). Hydrolysis of the compound by allowing to stand in 2 N KOH for 20 hr under a nitrogen atmosphere gave caffeic acid, which was identified by paper chromatography, UV, NMR and IR. Methylated products (IV) with dimethylsulfate were fractionated on a silicic acid column and yielded oily substance, UV λmax nm (ε): 218 (12200), 235 (10500), 300 (sh) (11900), 327 (15800). The mass spectrum of this compound showed the molecular ion peak at m/e 352 and base peak m/e 191 MeO−

\( \text{MeO} - \text{CH=CH-C=O} \). The NMR spectrum in CDCl₃ suggested that trans-caffeic acid and malic acid constituted the compound III: 2.93 (2H, d, J=6 Hz, −CH₂−), 3.71 (3H, −COOMe), 3.77 (3H, s, −COOMe), 3.89 (6H, s, Ar-OMe), 5.61 (1H, t, J=6 Hz, −CH), 6.35 (1H, d, J=16 Hz, −CH), 6.75~7.20 (3H, m, Ar−H), 7.69 (1H, d, J=16 Hz, −H), 8.08 (1H, s, −COOMe). With these results described above, F1-compound (III) was identified as phaselic acid (trans-caffeoyl-L-malic acid).

Subsequently, by the paper chromatographic analysis in 2% acetic acid, the F11-compound was shown to be a mixture of two components Rf 0.30 (I) and 0.52 (II), so that a further isolation was carried out over a cellulose powder column. As the result, I and II were obtained as pure oil in yield 920 mg (0.010% yield) and
1078 mg (0.012% yield) respectively. I showed
\[ \alpha \delta \] = -30.9° (c = 0.5, H2O) and UV max
nm (\( \lambda \)): 218 (sh) (19100), 232 (sh) (15500), 292
(13500), 326 (14700). When the solution of
I in 6N HCl was heated and then shaken with
ether, a phenol reagent-positive and nin-
hydrin-positive substance was obtained from
the water soluble portion, and identified as
3-(3,4-dihydroxyphenyl)-L-alanine (L-dopa)
by its mp 278-280°C, specific rotation \[ \alpha \delta \] = -9.75°
(c = 1.0, 1N HCl) and behaviors on paper-
chromatography. Besides, I was methylated
with dimethylsulfate and chromatographed
over silicic acid. Elution with 10% ethyl
acetate in chloroform yielded a crystal (V),
mp 152.0-153.0°C, \[ \alpha \delta \] +137° (c = 1, CHCl3).
Doragendorff’s reaction was positive. This
result indicates the presence of the amine
group. Anal. Found: C, 64.36; H, 6.59; N,
3.16, Caled. for C23H2,NO,: C, 64.49; H,
6.31; N, 3.04%. In the mass spectrum of V,
M+ ion peak was observed at m/e 429.
Furthermore fragment ions corresponding to
were observed at m/e 206, 191 and 151 (base peak).
The IR spectrum revealed absorption bands
at 3250, 1595, 1540, 1265 (>NH), 1730 (_\( \delta \)_),
1645, 970 (_\( \delta \)_), 840 and 800
\( \delta \) cm⁻¹. The UV spectrum in EtOH
showed an absorption maximum at 220, 233,
290 and 320 nm (\( \varepsilon \) = 22200, 24200, 19100 and
22200). The NMR spectrum (CDCl3) of V
indicated four Ar–OMe (3.77, 3H; 3.81, 3H;
3.88, 6H), one- COOMe (3.71, 3H), six Ar-H
(6.5-7.2, 6H, m), C=C (6.28, 1H, d, J =
16 Hz and 7.56, 1H, d, J = 16 Hz), >NH (6.13, 1H,
d, J = 8 Hz), –CH (4.99, 1H, dt, J = 8 and 5 Hz)
and –CH3 (3.10, 2H, d, J = 5 Hz). Hydro-
genation of I over palladium-black, followed
by methylation with dimethylsulfate, furnished
pentamethyl dihydrocompound (VI), mp 103.0
–103.5°C, [\( \alpha \delta \)] +56° (c = 1.0, CHCl3) and its
structure was determined by MS, NMR and
IR spectrum. When refluxed in 6N HCl,
V afforded O,O’-dimethylhydrocaffeic acid,
mp 95-96°C. An oxidation product of V,
was identified as veratic acid. From these
data, the structure of I was elucidated to be
N-trans-caffeoyl-3-(3,4-dihydroxyphenyl) -L-al-
anine.

On the other hand, oily substance II, ex-
hibited [\( \alpha \delta \)] = 28.7° (c = 0.57, H2O) and UV
\( \lambda \) max nm (\( \lambda \)): 288 (6700), 320 (5500), which
quite differed from that of I. Methylation of
II with dimethylsulfate afforded pentamethyl
compound (VII), [\( \alpha \delta \)] = 69° (c = 2, CHCl3).
The mass spectrum revealed m/e 429 (M+)
with a fragmentation pattern similar to that of
V. Additional fact suggesting the absence of a
trans-double bond was obtained from no
signal at 980 cm⁻¹ in the IR spectrum, and
these results led us to postulate that VII might
be a geometrical isomer (cis-form) of V. This
validity was given by the NMR spectrum;¹⁰
the signals at 5.89 (1H, d, J = 15 Hz) and 6.61
(1H, d, J = 15 Hz), which were assigned to cis-
double bond by comparing with the spectra of
trans- and cis-p-methoxy-cinnamic acids. Final-
ly, a hydrogenated product of VII on palla-
dium-black was compatible with dihydro
compound, mp 103.5-104.0°C, [\( \alpha \delta \)] +60.0°
(c = 1, CHCl3), Anal. Calcd. for C23H29NO,: C,
64.04; H, 6.73; N, 3.25%. Found: C, 64.04;
H, 6.73; N, 3.25%. The spectral data of its
MS, IR, UV and NMR were completely iden-
tical with those of VI. Thus, the structure of
II was established to be N-cis-caffeoyl-3-(3,4-
dihydroxyphenyl)-L-alanine.

Although several N-acylamino acids have
been found in nature,⁶,⁷ L-dopa conjugates
such as clovamides are the first findings, and
a interest is to assume that L-dopa, as a drug
against Parkinsonism,⁸ may be released from
them by a biological system.
REFERENCES