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CAFFEOYLQUINIC ACIDS FROM *PTEROCAULON VIRGATUM* AND *PLUCHEA SAGITTALIS*

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Key Word Index—*Pterocaulon virgatum*; *Pluchea sagittalis*; Compositae; caffeoylquinic acids; 3,4-dicaffeoylquinic acid; isochlorogenic acid.

Pterocaulon virgatum (L.) DC. and *Pluchea sagittalis* (Lam.) Cabrera (common name 'lucera') are two Compositae widely distributed in the northeastern region of Argentina [1]. They are used in folk medicine due to their tonic, sour and digestive properties ([2, 3]; Bowes, N., personal communication). Infusions of *P. sagittalis* have been described as colagogue and choleretic when given to patients with liver and gall-bladder problems [4].

In a previous paper we have reported the isolation of a flavone from *P. sagittalis* [5]. The present paper reports the isolation of 3,4-dicaffeoylquinic acid (yield 1 %) from *P. virgatum* and isochlorogenic acid (yield 0.2 %) (a mixture of 3,4-; 4,5- and 3,5-dicaffeoylquinic acid) from *P. sagittalis*. This is the first report of 3,4-dicaffeoylquinic acid as a single product; it always occurs in other sources with its isomers.

Caffeic acid and its esters have proved to increase bile flow in rats [6, 7]. The medicinal properties of these plants are presumably related to the high content of caffeic acid esters found in them.

EXPERIMENTAL

Aerial parts were collected in Concepción del Uruguay, Province of Entre Ríos, Argentina. Voucher specimens are deposited in the University Herbarium (Museo de Botánica, Universidad de Buenos Aires).

Extraction. Air-dried ground material of *P. virgatum* and *P. sagittalis* was extracted at room temp. with 25 % aq. MeOH. The aq. MeOH extracts were evapd to dryness, taken into hot water and partitioned with petrol, $CHCl_3$ and Et_2O.

Isolation of 3,4-*dicaffeoylquinic acid.* A ppt. appeared in the interphase H_2O–$CHCl_3$ (yield 1 %). The investigation of this ppt. by TLC showed it to be a single product which was identified as 3,4-dicaffeoylquinic acid by UV, IR, NMR, $[\alpha]_D^{20}$ and hydrolysis products.

3,4-*Dicaffeoylquinic acid.* Faint grey amorphous powder, mp 194–195°. UV: pale blue. UV/NH_3: greenish yellow. Colour reactions: NH_3: green; $FeCl_3$: grey. PC Whatman 1:2 % HOAc, H_2O and *n*-BuOH–HOAc–H_2O (4:1:5); R_f: 0.17, 0.10 and 0.80, respectively. TLC, $KHSO_4$–Si gel (1:14) in Et_2O–EtOAc (6:4); R_f: 0.64 [8]. (Found: C, 53.4; H, 5.48. $C_{25}H_{24}O_{12} \cdot 3H_2O$ requires: C, 52.7; H, 5.27 %). UV λ_{max}^{MeOH} nm: 233 sh, 243, 297 sh, 326. IR γ_{max}^{KBr} cm^{-1}: 3350, 2975, 2630, 2300, 1710, 1615, 1530, 1450, 1375, 1270, 1170, 1105, 1055, 975, 910, 850, 815, 780. ^{1}H NMR (60 MHz, C_5D_5N using TMS as internal standard): δ 7.30 (2H, *d*, $J = 12$ Hz; Hβ caffeic acid), 6.80 (2H, *d*, $J = 18$ Hz, Hα caffeic acid), 6.60 (4H, *s*, H2, 6 caffeic acid), 5.95 (2H, *d*, $J = 12$ Hz, H5 caffeic acid), 5.75 (1H, *m*, H3 quinic acid), 5.10 (1H, *m*, H4 quinic acid), 4.30 (1H, *m*, H5 quinic acid), 2.15 (4H, *m*, H2, 6 quinic acid). $[\alpha]_D^{20}$ −307 (MeOH, *c* 0.4).

Isolation of isochlorogenic acid. The Et_2O extract of *P. sagittalis* was passed through a column packed with Si gel PF 254 and eluted with mixtures of $CHCl_3$ and MeOH. The $CHCl_3$–MeOH eluates afforded by concn a yellow substance (yield 0.2 %) which was identified as isochlorogenic acid by TLC, IR, UV and its hydrolysis products by comparison with an authentic sample.

Isochlorogenic acid. Faint yellow amorphous powder. UV: pale blue. UV/NH_3: greenish yellow. Colour reactions: NH_3: green; $FeCl_3$: grey. PC Whatman 1:2 % HOAc, H_2O and *n*-BuOH–HOAc–H_2O (4:1:5); R_f: 0.17, 0.10, 0.80, respectively. (Found: C, 49.9; H, 5.70. $C_{25}H_{24}O_{12} \cdot 5H_2O$ requires: C, 49.5; H, 5.6 %). UV λ_{max}^{MeOH} nm: 235, 245, 295 sh, 328. IR γ_{max}^{KBr} cm^{-1}: 3350, 2875, 2925, 2350, 1700, 1610, 1520, 1460, 1380, 1290, 1160, 1100, 980, 930, 850, 780. $[\alpha]_D^{20}$ −153 (MeOH; *c* 0.49). TLC, $KHSO_4$–Si gel (1:14) in Et_2O–EtOAc (6:4) showed 3 spots R_f: 0.75, 0.64 and 0.54 corresponding to 3,5-; 3,4- and 4,5-dicaffeoylquinic acids, respectively. In all other solvents tested isochlorogenic acid was not resolved into its 3 isomers.

Quantitative hydrolysis. Both 3,4-dicaffeoylquinic and isochlorogenic acids were submitted to alkaline hydrolysis with 10 N KOH at room temp. for 30 min. Quinic acid was determined using the thiobarbiturate acid test described by Levy and Zucker [9]. Caffeic acid was determined by two methods: the one described by Nichiforescu–Coucou for *o*-diphenols with Arnow's reagent [10], and by determining the absorbance of combined caffeic acid at 327 nm. A calibration curve made with caffeic acid at different concns (1–6 μg/ml) was used. The sample (3 mg) was dissolved in H_2O and diluted to various concns.

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REFERENCES

1. Sorarú, S. B. and Bandoni, A. L. (1978) *Plantas de la Medicina Popular Argentina*, p. 41. Editorial Albatros, Buenos Aires.
2. Hieronymus, J. (1882) *Bol. Acad. Nac. Cienc., Córdoba, Argent.* 4, 343.
3. Parodi, D. (1881) *Ensayo de Botánica Médica Argentina Comparada*, Thesis, p. 48. P. Coni, Buenos Aires.
4. Bonorino Udaondo, C., Goñalons, G. P., Basile, A. R., Zunino, H. and Lacour, J. J. (1937) *Bol. Acad. Nac. Med. Buenos Aires* **20**, 441.
5. Martino, V. S., Ferraro, G. E. and Coussio, J. D. (1976) *Phytochemistry* **15**, 1086.
6. Czok, G., Midani, W. and Fiuke, R. I. (1971) *Colloq. Int. Chim. Cafes* **5**, 408.
7. Czok, G. and Schulze, P. J. (1973) *Z. Ernaehrungwiss.* **12**, 224.
8. Scarpati, M. L. and Guiso, M. (1963) *Ann. Chim.* **53**, 1315.
9. Levy, C. C. and Zucker, M. (1960) *J. Biol. Chem.* **235**, 2418.
10. Nichiforesco, E. and Coucou, V. (1965) *Ann. Pharm. Fr.* **23**, 419.