*Phytochemistry*, Vol. 25, No 1, pp. 167-170, 1986.
Printed in Great Britain.

0031 9422/86 $3.00 + 0.00


# DITERPENOIDS FROM *CAESALPINIA PULCHERRIMA*

DAVID D. MCPHERSON, CHUN-TAO CHE, GEOFFREY A. CORDELL*, D. DOEL SOEJARTO, JOHN M. PEZZUTO and HARRY H. S. FONG

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, IL 60612, U.S.A.

(*Received* 25 *April* 1985)

**Key Word Index**—*Caesalpinia pulcherrima*; Leguminosae; diterpenoids; vouacapen-5α-ol derivatives.

**Abstract**—Three new furanoditerpenoids of the caesalpin-type have been isolated from the roots of *Caesalpinia pulcherrima*. The structures of these compounds, vouacapen-5α-ol, 6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol and 8,9,11,14-didehydrovouacapen-5α-ol, were elucidated through interpretation of their spectral data. Sitosterol was also obtained.

## INTRODUCTION

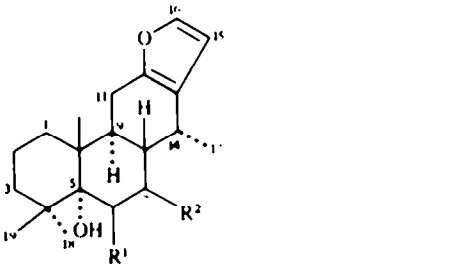
*Caesalpinia pulcherrima* Swartz, a tropical ornamental shrub, has been used as an abortifacient and an emmenagogue in folk medicine [1]. We have reported previously on the structures of some peltogynoids and homoisoflavonoids from the stems of this plant [2]. Further investigation of the root led to the isolation of four terpenoids. This paper presents the isolation and characterization of three new caesalpin-type diterpenoids, vouacapen-5α-ol (1), 6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol (2) and 8,9,11,14-didehydrovouacapen-5α-ol (3), together with sitosterol.

## RESULTS AND DISCUSSION

A chloroform extract of the roots of *C. pulcherrima* was chromatographed on silica gel to afford four crystalline compounds.

Vouacapen-5α-ol (1), showed a molecular ion at *m/z* 302 for a $C_{20}H_{30}O_2$ compound. The UV absorption at 220 nm along with an IR absorption at 1510 $cm^{-1}$ attested to the presence of a 2,3-disubstituted furan ring, and this was further supported by a pair of doublets ($J$ = 2 Hz) at δ6.18 and 7.23 in its $^1$H NMR spectrum (Table 1). The presence of a hydroxyl group was clear from the IR spectrum ($\nu_{max}$ 3592 $cm^{-1}$) and a mass fragment at *m/z* 284 $[M - H_2O]^+$. The hydroxyl group was not readily acetylated under normal conditions, suggesting the presence of a tertiary hydroxy at C-5, as in all other caesalpins that had previously been isolated from *Caesalpinia* spp. [3-9]. Hence compound 1 was considered to be a tricarbocyclic furanoditerpenoid and was likely to be related to the vouacapane skeleton [10].

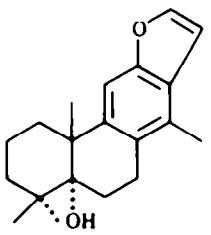
The $^{13}$C NMR spectrum of 1 (Table 2) displayed all twenty carbon signals. Four signals in the downfield region were typical for the carbons of a furan ring [11]. The only oxygenated $sp^3$ carbon (δ76.8, quaternary) observed in the spectrum was assigned to C-5. Thus, other positions on rings A and B were non-oxygenated; as was position C-14, since the 17-methyl signal was a doublet in the $^1$H NMR spectrum. Assignment of all carbon resonances was made possible by correlation with the chemical shifts for methyl vinhaticoate [11] using appropriate correction factors for 5α-hydroxylation on steroidal skeletons [12]. Examination of the high field $^1$H NMR spectrum (Table 1) revealed four groups of signals. In the upfield region, three singlets and one doublet ($J$ = 7 Hz) were ascribed to the methyl protons.



1 $R^1 = R^2 = H$

2 $R^1$ = —O—C(=O)—CH=CH—C$_6$H$_5$

$R^2$ = OH



3

*To whom correspondence should be addressed.

Table 1. $^1$H NMR data of compounds 1, 2 and 3 (360 MHz, $CDCl_3$, TMS as int. standard)*

| H | 1 | 2 | 3 |
|---|---|---|---|
| $1_\alpha$ | | 1.68 *br d*† | 1.98 *m*† |
| $1_\beta$ | 1.18 *br d* (14) | 1.17 *br d* (12) | 1.24 *br d* (13) |
| $2_\alpha$ | | 1.54 *br d*† | 1.70 *m*† |
| $2_\beta$ | | 1.65 *br d*† | 1.83 *m*† |
| $3_\alpha$ | | 1.76 *br d*† | 2.04 *m*† |
| $3_\beta$ | 1.15 1.85 *m* | 1.54 *br d*† | 1.83 *m*† |
| $6_\alpha$ | | 5.65 *d* (4) | 2.22 *ddd* (9,9,14) |
| $6_\beta$ | | | 2.05 *m* |
| $7_\alpha$ | | 4.38 *dd* (3.5, 11) | 2.89 *dd* (5.5,9) |
| $7_\beta$ | | | |
| 8 | | 1.98 *ddd* (5,11,12) | — |
| 9 | 2.44 *br dd* (10,12) | 2.45 *dt* (9,12) | — |
| $11_\alpha$ | 2.35 *m* | 2.54 *br d* (9) | 7.32 *s* |
| $11_\beta$ | | | — |
| 14 | 2.58 *dq* (4,7) | 3.05 *dq* (6,7) | - |
| 15 | 6.18 *d* (2) | 6.20 *d* (2) | 6.73 *d* (2) |
| 16 | 7.23 *d* (2) | 7.23 *d* (2) | 7.53 *d* (2) |
| 17 Me | 1.01 *d* (7) | 1.07 *d* (7) | 2.38 *s* |
| 18 Me | 1.05‡ *s* | 1.21‡ *s* | 1.05‡ *s* |
| 19 Me | 1.07‡ *s* | 1.45‡ *s* | 1.15‡ *s* |
| 20 Me | 0.94 *s* | 1.09 *s* | 1.34 *s* |
| OH | 1.34 *s* | 1.80 *s* | 1.42 *s* |
| Cinnamate | | | |
| H-2' | - | 6.44 *d* (16) | — |
| H-3' | — | 7.72 *d* (16) | — |
| aromatic | | 7.38 *m* | — |
| | | 7.53 *m* | |

*Coupling constants (Hz) in parentheses.

†Assignments made equivocally based on similar structures [14,15].

‡Assignment may be reversed.

Table 2. $^{13}$C NMR data of compounds 1, 2 and 3 (90.8 MHz, $CDCl_3$, TMS as int. standard)

| Carbon | 1* | 2* | 3† |
|---|---|---|---|
| 1 | 32.5 | 35.0 | 33.0 |
| 2 | 18.2 | 18.1 | 18.9 |
| 3 | 36.4 | 37.8 | 36.2 |
| 4 | 38.4 | 39.3 | 37.9 |
| 5 | 76.8 | 76.8 | 75.8 |
| 6 | 25.7 | 73.6 | 24.8 |
| 7 | 22.3 | 69.2 | 23.8 |
| 8 | 34.5 | 37.9§ | 125.4‡ |
| 9 | 37.6 | 37.2§ | 144.5 |
| 10 | 41.2 | 41.1 | 43.7 |
| 11 | 24.8 | 21.8 | 104.8 |
| 12 | 149.8 | 149.2 | 153.7 |
| 13 | 122.6 | 120.0 | 128.3‡ |
| 14 | 31.5 | 27.3 | 126.8‡ |
| 15 | 109.6 | 109.7 | 105.0 |
| 16 | 140.3 | 140.5 | 144.1 |
| 17 | 17.5‡ | 17.3‡ | 27.7 |
| 18 | 28.1 | 27.7 | 29.3 |
| 19 | 24.8 | 25.5 | 24.8 |
| 20 | 17.1‡ | 17.1‡ | 15.8 |
| Cinnamate | | | |
| 1' | – | 167.4 | |
| 2' | – | 118.0 | |
| 3' | - | 145.9 | — |
| 4' | — | 134.2 | — |
| 5', 9' | -- | 128.9 | — |
| 6', 8' | — | 128.2 | — |
| 7' | — | 130.5 | – |

*Insensitive nuclei enhanced by polarization transfer (INEPT) method was used to aid assignments.

†Attached proton test (APT) method was used to aid assignments.

‡§Assignments marked by the same sign in the same column may be interchanged.

Between $\delta$1.1 and 1.9, mostly poorly resolved signals integrating for twelve protons were observed. One of them, a singlet at $\delta$1.34 exchangeable with deuterated water, was assigned to a hydroxyl group. A broad doublet at $\delta$1.18 was possibly due to the H-1$\beta$. Other signals in this region were not readily assignable. Double irradiation experiments allowed the assignment of H-9, H-14, and H-11 in the range $\delta$2.3 2.6. The H-9 signal appeared as a broad doublet of doublets, showing large coupling constants with H-8 and H-11$\beta$ (10 and 12 Hz), but the two H-11 proton signals were indistinguishable. On the other hand, the H-14 proton was observed at $\delta$2.58 (doublet of quartets), coupled to both the 17-methyl ($J$ = 7 Hz) and the H-8 ($J$ = 4 Hz). These coupling constants indicated that H-8 and H-9 were axial, while H-14 was equatorial. Further downfield, a pair of doublets ($J$ = 2 Hz) were assigned to the $\alpha$- and $\beta$-furan protons. Vouacapen-5$\alpha$-ol was therefore determined to have the caesalpin structure **1**. The compound was thus named in order to distinguish it from a reduction product of vouacapenic acid, vouacapenol, which should be regarded as vouacapen-18-ol [10]. Compound **1** is also related to vinhaticol [10], which is preferably named as vouacapen-19-ol.

6$\beta$-Cinnamoyl-7$\beta$-hydroxy-vouacapen-5$\alpha$-ol (**2**) had a $C_{29}H_{36}O_5$ molecular formula. A vouacapenol skeleton was suggested when the spectral properties of this compound were compared with **1**. The UV spectrum, in addition to a maximum at 220 nm corresponding to a furan ring, exhibited $\lambda_{max}$ at 210 (sh), 225, and 280 nm. This spectrum, along with the IR $\nu_{max}$ 1717, 1642, 980 cm$^{-1}$, and the $^1$H NMR and $^{13}$C NMR data (Tables 1 and 2), and a base-peak in the mass spectrum at $m/z$ 131 confirmed the presence of a *trans*-cinnamoyl moiety. In the $^1$H NMR spectrum, two signals for protons on oxygen-bearing carbons were observed ($\delta$ 4.38 and 5.65). A series of double irradiation experiments defined the relationship between these two protons (H-7 and H-6), as well as those between several other proton signals (H-8, H-9, H-11, H-14 and 17-methyl). The cinnamoyl ester was assigned to the C-6 position because H-6 was coupled only to H-7. An equatorial axial relationship between H-6 and H-7 was inferred from the coupling constant (4 Hz),

implying that both the 6-cinnamoyl and 7-hydroxyl groups had a β-stereochemistry. Thus, the relative stereochemistry at position 6 of **2** was different from other known 6,7-disubstituted caesalpins, all of which had previously shown large coupling constants (10 Hz) between H-6 and H-7 [7]. Examination of the $^{13}C$ and the $^1H$ NMR data aided in the stereochemical determination of the other asymmetric centres. Using podocarpan-12-ol and methyl vinhaticoate as reference compounds [11, 13], the chemical shifts of the carbons of rings A and B fitted well with those reported in the literature [12] (using cholestane as a model) for the presence of a 5α-hydroxyl substituent. The hydroxyl group attached to C-5 was therefore axial. To determine the configuration at C-8, C-9 and C-14, the high-field $^1H$ NMR was found to be useful. The proton at C-8 was placed in an axial orientation on the basis of its large coupling constants (11 Hz) with H-7 and H-9. It followed that H-9 was also axial. Because the H-14 to H-8 coupling constant was only moderate (5 Hz), H-14 was deduced to be equatorial. The complete stereochemical assignments are shown in **2**.

8,9,11,14-Didehydrovouacapen-5α-ol (**3**), a $C_{20}H_{26}O_2$ compound, ([M]$^+$ at *m/z* 298) possessed a benzofuran moiety (UV: $\lambda_{max}$ 215, 251, 258 sh, 282, 292 nm; $^1H$ NMR: δ6.73, *d*, *J* = 2 Hz, 7.53, *d*, *J* = 2 Hz; $^{13}C$ NMR: eight aromatic carbons as shown in Table 2). An aromatic methyl signal (17-methyl) at δ2.38 replaced the upfield methyl signal in the spectra of **1** and **2**, and an $A_2BX$ system, with proton signals centred at δ2.05, 2.22, and 2.89, was assigned to the protons at C-6 and C-7. Irradiation at δ2.89 (7-methylene) collapsed a *ddd* at δ2.22 (H-6α) to a doublet, $J_{6\alpha,6\beta}$ = 14 Hz, and also reduced in complexity the region of δ2.0. A sharp doublet (*J* = 14 Hz) now appeared in the midst of the envelope, indicating it to be the H-6β proton. In the upfield region of the spectrum, a broad doublet at δ1.24 was assigned to H-1β. Other methylene protons, resonating as multiplets, were equivocally assigned by using the reported values of other terpenoids [14, 15]. All twenty carbons were observed in the $^{13}C$ NMR spectrum (Table 2), one of them being a quaternary oxygenated $sp^3$ carbon. Resistance of the compound to acetylation with acetic anhydride again supported the assignment of a tertiary hydroxy group at C-5, indicating other positions on rings A and B to be unsubstituted. The carbon spectrum was assigned by comparison with other caesalpins, and by comparison with the aromatic diterpenoid 12-hydroxy-8,11,13-podocarpatriene [16]. 8,9,11,14-Didehydrovouacapen-5α-ol (**3**) is therefore a benzofuran derivative of vouacapen-5α-ol. Although it is known that caesalpins can be transformed into benzofurans by the action of mild acid [6], **3** is a natural product since it could be detected in the initial chloroform extract of the root material without acid treatment.

Compound **2** was found to be cytotoxic in the KB and P-388 *in vitro* test systems [17–19], displaying $ED_{50}$ values of 1.8 μg/ml and 3.5 μg/ml, respectively.

## EXPERIMENTAL

Mps (Kofler hot-stage): uncorr; NMR (Nicolet NMC-360): 360 MHz for proton resonances and 90.8 MHz for carbon resonances; Low-resolution MS: 70 eV. The plant material was obtained as described previously [2].

*Extraction and fractionation.* Air-dried and ground root material of *C. pulcherrima* Swartz (4.6 kg) was extracted with $CHCl_3$. Concn of the extract *in vacuo* afforded a residue (240 g) which was dissolved in $CHCl_3$ and chromatographed on a column of silica gel 60 (5 kg) packed in the same solvent. A total of 70 fractions (2 l each) were collected as the solvent was progressively changed to increasingly polar mixtures of $CHCl_3$–MeOH, 99:1, 95:5, and 90:10.

*Isolation of vouacapen-5α-ol* (**1**). Fractions 5–7 (3.6 g) obtained from the above column were dissolved in $CHCl_3$–MeOH (9:1) and allowed to stand at 4°. Colourless needles formed and were recrystallized from a $CHCl_3$–MeOH mixture to afford 375 mg **1**, mp 98–100°; $[\alpha]_D^{25}$ + 51° ($CHCl_3$, *c* 0.1); UV $\lambda_{max}^{EtOH}$ nm (log ε): 220 (3.98); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3592, 2933, 1650, 1510; $^1H$ NMR ($CDCl_3$): see Table 1; $^{13}C$ NMR ($CDCl_3$): see Table 2; MS *m/z*: 302 [M]$^+$ (36%), 284 (17), 269 (9), 202 (13), 199 (23), 171 (22), 145 (42), 133 (23), 109 (100).

*Isolation of 6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol* (**2**). Fractions 21–32 (55 g) from the above column afforded a ppt after standing at 4°. The ppt was collected and recrystallized twice from $CHCl_3$ to yield 275 mg **2**, as colourless prisms: mp 218–221°; $[\alpha]_D^{25}$ + 70° ($CHCl_3$, *c* 0.5); UV $\lambda_{max}^{EtOH}$ nm (log ε): 210 sh (4.24), 220 (4.47), 225 (4.39), 280 (4.49); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3580, 2935, 1717, 1711, 1642, 1510; $^1H$ NMR ($CDCl_3$): see Table 1; $^{13}C$ NMR ($CDCl_3$): see Table 2; MS *m/z*: 464 [M]$^+$ (9%), 398 (3), 283 (2), 232 (5), 147 (4), 131 (100), 103 (18).

*Isolation of 8,9,11,14-didehydrovouacapen-5α-ol* (**3**). Fractions 1–4 (6.5 g) of the above column were obtained as an oil. Separation was achieved on a silica gel column, eluting with solvent mixtures containing petrol and $CHCl_3$ of increasing polarity. Fractions of 100 ml each were collected. Fractions 10–30 (20 mg) were applied to prep. TLC plates (developed with $Et_2O$–$CHCl_3$, 1:49) to afford a colourless residue which was recrystallized from MeOH to afford **3** as needles (8 mg): mp 117–118°; $[\alpha]_D^{25}$ + 30° (EtOH, *c* 0.1); UV $\lambda_{max}^{EtOH}$ nm (log ε): 215 (4.57), 251 (4.02), 258 sh (3.94), 282 (4.08), 292 (4.10); IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3616, 3553, 2942, 1730, $^1H$ NMR ($CDCl_3$): see Table 1; $^{13}C$ NMR ($CDCl_3$): see Table 2; MS *m/z*: 298 [M]$^+$ (46%), 280 (63), 265 (43), 213 (51), 198 (100).

*Isolation of β-sitosterol.* Fractions 16–17 (6.9 g) afforded a colourless crystalline compound (0.3 g) after crystallization from a $CHCl_3$–MeOH mixture. The compound was identical in all respects (co-TLC, mp, $^1H$ NMR, MS) with an authentic sample of β-sitosterol.

*Biological activity. In vitro* cytotoxic tests were performed using KB and P-388 cell lines as described in the literature [17–19].

*Acknowledgements* This investigation was supported, in part, by grant CA 33047 from the Division of Cancer Treatment, National Institutes of Health, Bethesda, MD. One of us (DDM) was the recipient of a Predoctoral Fellowship awarded by the National Institutes of Health, 1982–1983. JMP is the recipient of a Research Career Development Award from the National Cancer Institute, 1984–1989. The Task Force on Indigenous Plants for Fertility Regulation, Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization (HRP Project 77918C) is thanked for the supply of plant material used in this study. The assistance of Dr. S. Balasubramanian, Department of Botany, University of Peradeniya, Sri Lanka, during the botanical field work and of Ms. S. Yeh of the University of Illinois in obtaining the NMR spectra is sincerely appreciated. We also gratefully acknowledge the NMR Laboratory of the Research Resources Center, University of Illinois at Chicago, for providing NMR instrumentation and assistance in the conduct of this work.

## REFERENCES


1. Quisumbing, E. (1951) *Medicinal Plants of the Philippines*, pp. 372-374. Manila Bureau of Printing.
2. McPherson, D. D., Cordell, G. A., Soejarto, D. D., Pezzuto, J. M. and Fong, H. H. S. (1983) *Phytochemistry* **22**, 2835.
3. Canonica, L., Jommi, G., Manitto, P., Pagnoni, U. M. and Pelizzoni, F. (1966) *Gazz. Chim. Ital.* **96**, 662.
4. Canonica, L., Jommi, G., Manitto, P., Pagnoni, U. M. and Pelizzoni, F. (1966) *Gazz. Chim. Ital.* **96**, 687.
5. Canonica, L., Jommi, G., Manitto, P., Pagnoni, U. M. and Pelizzoni, F. (1966) *Gazz. Chim. Ital.* **96**, 698.
6. Balmain, A., Birnbaum, K., Connolly, J. D. and Ferguson, G. (1967) *Tetrahedron Letters* **49**, 5207.
7. Balmain, A., Connolly, J. D., Ferrari, M., Ghisalberti, E. L., Pagnoni, U. M. and Pelizzoni, F. (1970) *Chem. Commun.* 1244.
8. Sengupta, P., Roy, S. and Das, K. G. (1970) *Chem. Ind. (London)* 534.
9. Purushothaman, K. K., Kalyani, K., Subramanian, K. and Shanmuganathan, S. (1981) *Indian J. Chem.* **20B**, 625.
10. King, F. E., Godson, D. H. and King, T. J. (1955) *J. Chem. Soc.* 1117.
11. Connolly, J. D., Orsini, F., Pelizzoni, F. and Ricca, G. (1981) *Org. Magn. Reson.* **17**, 163.
12. Eggert, H., VanAntwerp, C. L., Bhacca, N. S. and Djerassi, C. (1976) *J. Org. Chem.* **41**, 71.
13. Wahlberg, I., Almqvist, S. O., Nishida, T. and Enzell, C. R. (1975) *Acta Chem. Scand.* **29B**, 1047.
14. Bohlmann, F., Umemoto, K., Jakupovic, J., King, R. M. and Robinson, H. (1984) *Phytochemistry* **23**, 1800.
15. Bohlmann, F., Jakupovic, J., Schuster, A., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2317.
16. Nishida, T., Wahlberg, I. and Enzell, C. R. (1977) *Org. Magn. Reson.* **9**, 203.
17. Geran, R. I., Greenberg, N. H., MacDonald, M. M., Schumacher, A. M. and Abbott, B. J. (1972) *Cancer Chemother. Rep.* **3**, 1.
18. Pezzuto, J. M., Antosiak, S. K., Messmer, W. M., Slaytor, M. B. and Honig, G. R. (1983) *Chem.-Biol. Interact.* **43**, 323.
19. Arisawa, M., Pezzuto, J. M., Bevelle, C. and Cordell, G. A. (1984) *J. Nat. Prod.* **47**, 453.