Journal of Natural Products
Vol. 49, No. 4, pp. 561-569, Jul-Aug 1986

PULCHERRALPIN, A NEW DITERPENE ESTER FROM *CAESALPINIA PULCHERRIMA*

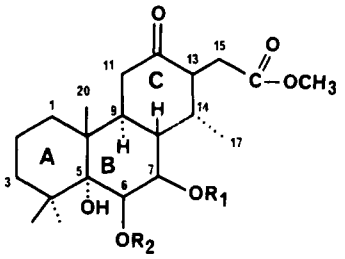
CHUN-TAO CHE, DAVID D. MCPHERSON, GEOFFREY A. CORDELL,* and HARRY H.S. FONG

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

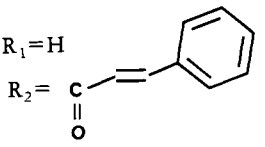
ABSTRACT.—From the stems of *Caesalpinia pulcherrima*, the new cassane-type diterpene ester pulcherralpin (**1**) has been isolated and characterized. The structure of **1** was established through spectroscopic studies, including the ^{1}H-^{1}H homonuclear and ^{1}H-^{13}C heteronuclear correlation studies.

As part of our continuing investigation of potential fertility-regulating and antitumor agents from plant sources, we have previously reported on the isolation and structure elucidation of peltogynoids, homoisoflavonoids, caesalpins, a benzoquinone, and a chalcone from *Caesalpinia pulcherrima* Swartz (Leguminosae) (1,2), a tropical plant used in Southeast Asia as an abortifacient and emmenogogue (3). Several of these compounds displayed cytotoxic effects (1,2). Further study of the stem plant part has led to the isolation and structure elucidation of a cassane-type diterpene ester pulcherralpin (**1**).

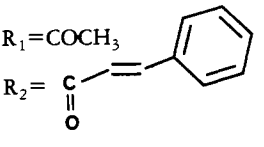
Pulcherralpin (**1**, 0.0015% yield), isolated as colorless needles, mp 243-246°, $[\alpha]D$ +22.5 ($CHCl_3$, *c* 0.27), displayed a molecular ion at *m/z* 512, analyzing for $C_{30}H_{40}O_7$. Strong ir absorptions at 1735 and 1250 cm^{-1}, along with a nmr singlet (3H) at δ 3.66, indicated the presence of a methoxy carbonyl group. The presence of a *trans*-cinnamoyl moiety was inferred from the ^{1}H-nmr (Table 1) and ^{13}C-nmr spectra (Table 2), the uv spectrum (λ max 209, 220, 226, 280, nm), and the ir absorptions (ν_{max} 1715, 1635, 980 cm^{-1}). The ir spectrum also displayed an additional carbonyl stretching signal at 1725 cm^{-1}, as well as hydroxy absorptions at 3350 and 3575 cm^{-1}. Hence, compound **1** was considered to be a tricarbocyclic diterpenoid, and the



1 R_1=H

R_2= 

2 R_1=$COCH_3$

R_2= 

3 R_1=R_2=H

TABLE 1. ^{1}H nmr Chemical Shifts of Pulcherralpin (**1**), Pulcherralpin Acetate (**2**), and the Hydrolysis Product **3**

Proton	Compound			
	1		**2**	**3**
	360 MHz, Pyridine-d_5	360 MHz, $CDCl_3$	200 MHz, Pyridine-d_5	200 MHz, Pyridine-d_5
1α	1.24, br d (12)			
1β	1.81, br d (12)			
2α	1.72, m	1.1-2.2	1.1-2.2	1.1-2.2
2β	1.45, m			
3α	2.15, br t (13)			
3β	1.08, br d (13)			
6	6.34, d (4)	5.60, d (3.5)	6.27, d (4)	4.67, d (4)
7	4.80, dd (4, 11)	4.30, dd (3.5, 11)	6.03, dd (4, 11)	4.59, dd (4, 11)
8	2.72, ddd (3, 11.5, 12)	2.2-2.3	2.78, m	2.58, ddd (4, 11, 12)
9	2.99, ddd (7, 11.5, 12)	2.45, dt (9, 11)	2.90, m	2.86, ddd (7, 11, 12)
11a,b	2.48, m	2.26, d (9)	2.45, m	2.40, m
13	3.43, ddd (3, 5, 9)	3.12, dd (4.5, 9)	3.36, ddd (3, 5, 10)	3.33, ddd (3, 5, 10)
14	3.10	2.58, m	2.95, m	3.15, m
15a	2.24, dd (5, 17)	2.12, dd (5, 16)	2.25, m	2.35, dd (5, 17)
15b	3.13, dd (9, 17)	2.86, dd (9, 16)	3.05, dd (10, 16)	3.11, dd (9, 17)
17-H_3	0.92, d (7)	0.75, d (9)	0.79, d (7)	0.89, d (7)
18-H_3	1.44, s	1.21, s	1.29, s	1.29, s
19-H_3	1.41, s	1.05, s	1.32, s	1.61, s
20-H_3	1.64, s	1.48, s	1.63, s	1.77, s
OCH_3	3.59, s	3.66, s	3.62, s	3.64, s
OH	6.55, br s	1.53, s		4.79, s
	7.65, s	1.85, s		5.80, bs
				6.03, bs
$COCH_3$	—	—	2.12, s	—
Cinnamate				
2′	6.99, d (16)	6.50, d (16)	7.06, d (16)	—
3′	8.08, d (16)	7.77, d (16)	8.12, d (16)	—
5′,9′	7.65, m	7.57, m	7.75, m	—
6′,7′,8′	7.38, m	7.41, m	7.42, m	—

TABLE 2. ^{13}C-nmr Chemical Shifts of Pulcherralpin (**1**)

Carbon	Values in ppm		
	90.8 MHz, Pyridine-d_5	90.8 MHz, $CDCl_3$	Estimated Values from the INKA system
1	33.7	33.9	34.3±1.0
2	18.3	17.9	18.1±0.9
3	37.6	37.5	34.0±5.1
4	39.2	39.1	39.4±0.1
5	77.4	77.9	80.3±4.8
6	74.2	73.4	69.7±3.0
7	67.1	68.4	72.9±1.7
8	41.6	41.0	45.8±2.8
9	41.1	40.9	53.9±1.0
10	41.3	40.1	48.4±5.1
11	40.4	40.0	43.1±4.5
12	211.4	211.7	206.6±3.5
13	50.6	50.8	52.0±2.9
14	35.5	35.2	36.9±2.9
15	31.7	31.6	35.7±0.1
16	172.6	173.0	172.7±1.1
17	8.2	8.4	15.3±1.2
18	27.8	27.6	30.0±3.8
19	25.0	25.2	30.0±3.8
20	16.3	16.4	17.8±1.2
OCH_3	50.8	51.8	51.5±0.2
1′	166.3	167.3	167.2±0.9
2′	119.3	117.5	117.1±1.8
3′	144.4	146.2	144.7±0.7
4′	134.7	133.9	134.4±0.5
5′,9′	128.0	128.2	128.7±0.4
6′,8′	128.6	128.5	128.7±0.4
7′	130.0	130.5	127.6±1.1

spectral data suggested the presence of the cassane skeleton (4), containing a ketone, two ester groups, and two hydroxyl groups. The regio and stereo placement of these groups on the nucleus is the focus of this paper.

The ^{1}H-nmr spectra (Table 1) were recorded both in deuterated pyridine and chloroform. In pyridine, seven signals for a cinnamate were clearly shown in the downfield region, and its attached methine proton was observed at δ 6.34 as a doublet. The proton-proton correlation spectrum was particularly important in establishing the substitution pattern on the nucleus. Thus, slightly further upfield of the aromatic region, a doublet at δ 6.34 (H-6) was coupled to a doublet of doublets at δ 4.80 (H-7), which was further coupled with a complex signal at δ 2.72 (H-8). On acetylation with Ac_2O-pyridine, the H-7 signal of the acetate **2** experienced a downfield shift to δ 6.03. Therefore, the secondary hydroxyl group was assigned to C-7 and the cinnamoyloxy group, to C-6. Another hydroxy was placed at C-5 since no H-5 proton was observed in the spectrum and H-6 was coupled only to H-7. In order to confirm the presence of a cinnamoyl ester, compound **1** was subjected to alkaline hydrolysis with 0.1N NaOH in MeOH. The ^{1}H-nmr spectrum of the hydrolysis product **3** revealed two signals around the δ 4.6 region. A doublet at δ 4.67 was assigned to H-6 while a doublet of doublets at δ 4.59 was ascribed to H-7. The significant upfield shift of H-6 resonance compared to that of **1** (δ 6.34) is consistent with the removal of an ester group from the C-6 position. Furthermore, downfield signals corresponding to the cinnamate protons were absent from

the spectrum of **3**. Other features of the spectrum were comparable to those of **1** (Table 1).

With the aid of the homonuclear 2D-nmr spectrum (Figure 1), the H-8 signal in **1** was shown to be coupled with a complex at δ 2.99 (ddd, H-9) and a hidden signal at δ 3.10 (H-14), to which a secondary methyl group (17-H_3) was coupled. The H-9 signal was also observed to couple with a multiplet at δ 2.48, which collapsed to a broad singlet when H-9 was irradiated. This signal, integrating for two protons, was ascribed

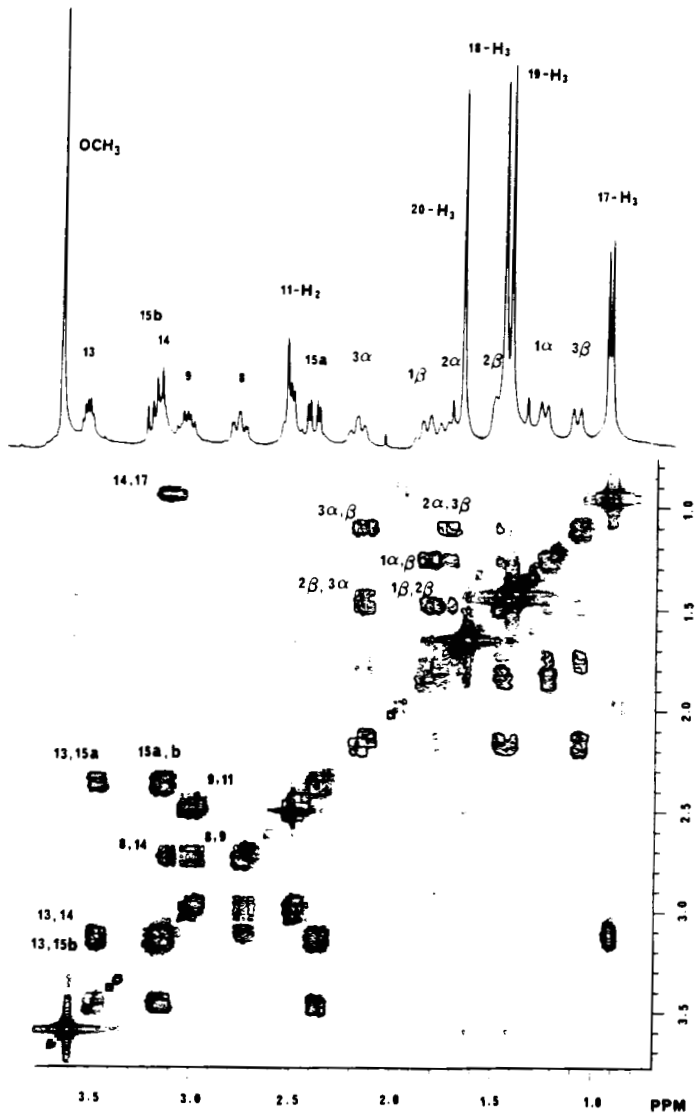


FIGURE 1. ^1H-^1H Homonuclear correlation spectrum (upfield region) of pulcherralpin (**1**).

to the 11-CH_2 group. The above results led to a carbon-proton sequence that could be accommodated only in rings B and C of a cassane skeleton. When determined in deuterated pyridine, the nmr signal for H-13 was observed at δ 3.43 as a ddd, coupling to H-14, H-15a, and H-15b. The two 15-CH_2 protons were found at δ 2.24 and 3.13 as doublet of doublets. In $CDCl_3$, however, H-15a, H-15b, and H-13 were observed as an AMX system with protons centered at δ 2.12, 2.86, and 3.12. The fact that H-13 has

very small coupling with H-14 may be explained by a dihedral angle of close to 90° between these protons as shown in a Dreiding model. These assignments were further confirmed by double irradiation experiments.

All 30 carbons of **1** were observed in the ^{13}C-nmr spectrum (Table 2). The downfield region consists of seven signals corresponding to a cinnamate group. In addition, two carbonyl singlets were present at δ 172.6 and 211.4, indicative of an ester (C-16) and a ketone (C-12), respectively. In the upfield region of the spectrum, the attached proton test (APT) technique and the ^{1}H-^{13}C 2D-nmr spectrum facilitated the assignment of the carbon signals. Thus, two nonoxygenated quaternary carbons were assigned to C-4 and C-10, leaving the oxygenated quaternary carbon to be attributed to C-5. Methylene signals due to C-1, C-2, C-3, and C-11, initially assigned on the basis of estimated values from related compounds using correction factors such as 5α-hydroxylation and 12-ketone substitution (5,6), were confirmed by 2D-nOe experiments as described subsequently. The C-11 signal could be distinguished from the others by its correlation with the 11-CH_2 proton signals in the ^{1}H-nmr spectrum. The last methylene peak was then assigned to C-15, which was observed to correlate with two magnetically unequivalent protons ascribed to H-15a and H-15b. Of the two oxygenated methine carbon signals, the one at more downfield position was assigned to C-6 while the other, to C-7. The spectrum also displayed four nonoxygenated methine carbons. The unequivocal assignment of each carbon has been achieved by the ^{1}H-^{13}C 2D-nmr results as shown in Figure 2. For example, the C-8 (δ 41.6) and C-9 (δ 41.1) signals were observed to correlate with the proton resonances at δ 2.72 and 2.99, respectively, which have unambiguously been determined to be H-8 and H-9. In a similar manner, assignments of C-13 and C-14 were also achieved. Finally, the five methyl groups in the

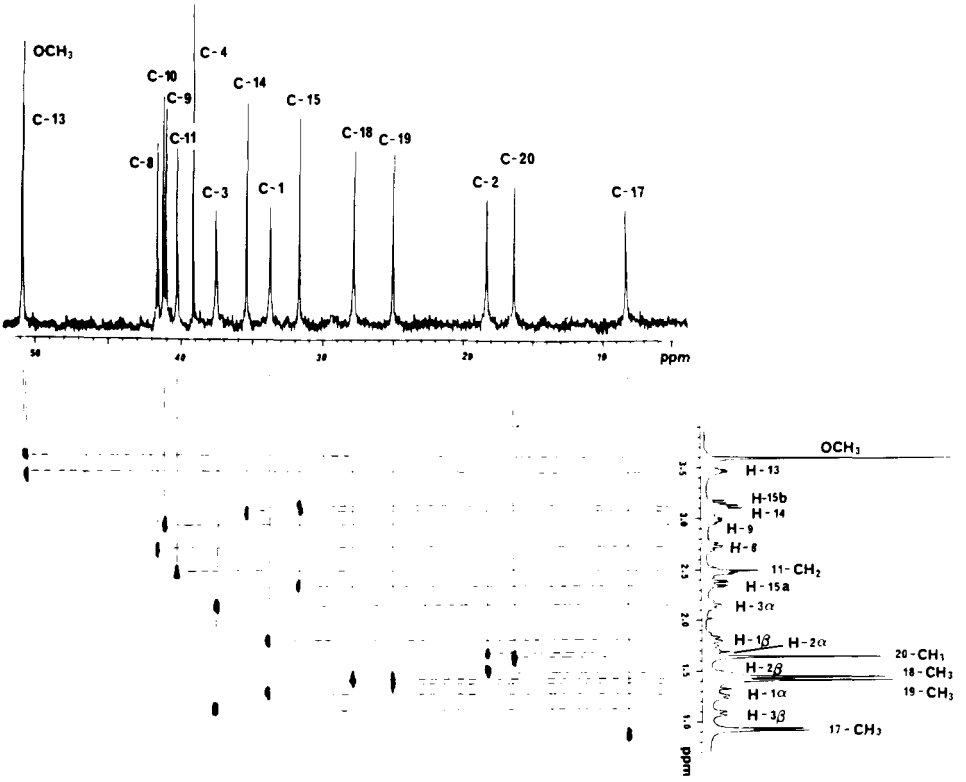


FIGURE 2. ^{1}H-^{13}C Heteronuclear correlation spectrum (upfield region) of pulcherralpin (**1**)

molecule could be assigned with reference to known compounds (7). Thus C-17 showed a very upfield resonance at δ 8.2, resembling C-17 in the caesalpins (2,7); it also correlates with an upfield proton doublet (δ 0.92) in the ^{1}H-nmr spectrum. Assignments of C-18, C-19, C-20 were supported by ^{1}H-^{13}C 2D-nmr and 2D-nOe spectra (Figures 2 and 3). A correlation signal in the 2D-nOe spectrum showing long-range coupling between CH_2-11 and CH_3-20 confirmed the assignment of the latter. Furthermore, the 2D-nOe spectrum permitted differentiation of CH_3-19 from CH_3-18, since a correlation signal between CH_3-20 and CH_3-19 was observed. In this way, the complete assignment of the carbon signals of **1** was achieved (Table 2). The location of a carbonyl group at C-12 was made with reference to the chemical shifts of the C-11 and C-13 carbons and attached protons; it was also based on the observation that the 11-methylene protons were not further coupled. Biogenetically, the C-12 position is always oxygenated in caesalpins thus far found in *Caesalpinia* plants (2,7-11).

In order to clarify ambiguity concerning the assignments of the ring-A methylene signals, particularly C-1 and C-3, efforts were made to obtain specific spectroscopic evidence. The long-range selective-INEPT method (12) was attempted, without success, to show the relationship between H-9 and C-1. However, a solution to the problem was

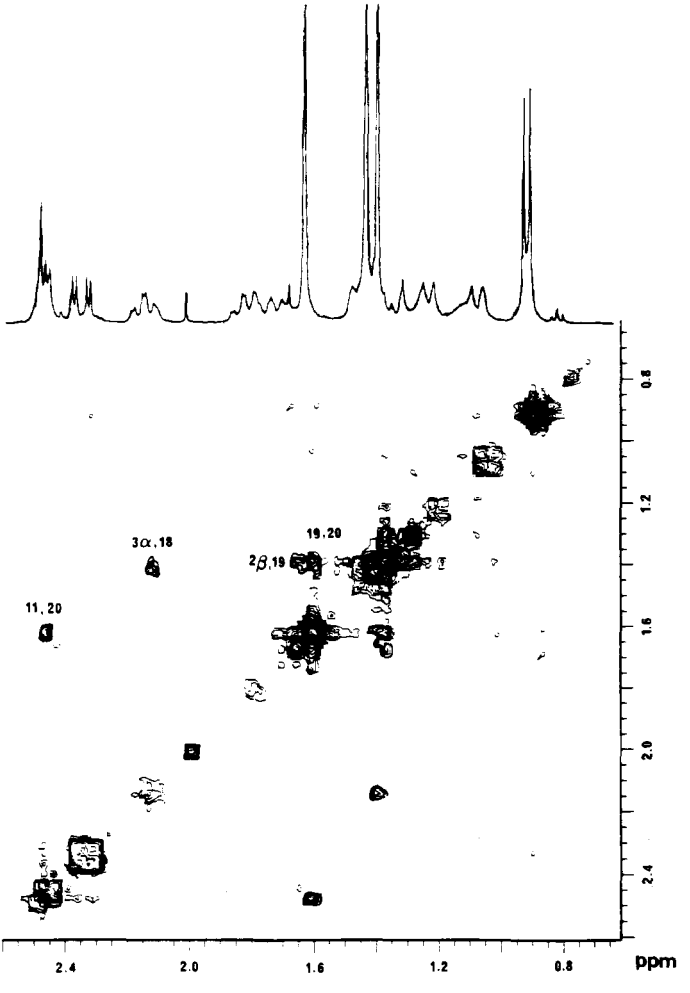


FIGURE 3. Two-dimensional nOe spectrum (upfield region) of pulcherrallpin (**1**).

obtained indirectly from a 2D-nOe experiment. As shown in Figure 3, H-3α could be located by a correlation signal with CH_3-18. This permitted assignment of C-3 (δ 37.6) and H-3β (δ 1.08) with confidence. Carbon-1 was then assigned to a signal at δ 33.7, which correlates with the two proton signals at δ 1.24 (H-1α) and 1.81 (H-1β). Finally utilizing the C-2 signal (δ 18.3), an unequivocal assignment of the CH_2 protons was made.

Additional evidence for the proposed structure of **1** was obtained from the mass spectrum, in which peaks at *m/z* 481 (M^+ -OCH_3), 439 (M^+ -CH_2COOCH_3), 364 (M^+ -cinnamic acid), 346 (364-H_2O), 331 (346-CH_3), 313 (331-H_2O), and 131 (base peak, cinnamate cation) were observed. This fragmentation pattern supports the presence of methoxycarbonylmethyl, cinnamate, and two hydroxyl functions in the molecule.

The chemical structure of **1** was further supported by a computer-generated carbon spectrum obtained from the INKA ^{13}C-nmr Data Base.[1] The results are included in Table 2 for comparison. It is clear that the estimated carbon chemical shifts are in good agreement with the observed values.

The relative stereochemistry of **1** was determined by the high-field nmr results. The H-7 proton shows a substantial coupling constant (11 Hz) with H-8 and is therefore axial. Likewise, H-9 couples to H-8 with a J value of 12 Hz, indicating a diaxial relationship. On the other hand, the relatively small coupling (4 Hz) between H-6 and H-7 is consistent with the assignment of an equatorial disposition for H-6. Irradiation of the 17-H_3 group resulted in the collapse of the multiplet for H-14 to a doublet, $J_{8,14}$=3 Hz, implying an equatorial orientation of H-14. Furthermore, the small coupling constant between H-13 and H-14 led to the conclusion that the former must be oriented in the (pseudo) equatorial position to form a dihedral angle of ca 90°.

The proposed *trans-anti-trans* stereochemistry with an axial sidechain at C-13 was supported by the cd curve which showed a positive carbonyl Cotton effect, as predicted by the octant rules (13) and by comparison with data for 12-oxo-abietanoates (14).

Compound **1** was thus determined to be a cassane-type diterpene for which we propose the trivial name pulcherralpin. Pulcherralpin is structurally related to the caesalpins found previously in *Caesalpinia* plants (2,7-11). Biogenetically, it may be related to the caesalpins through a common precursor, or they may be related through a series of enzymatic reduction, ring-closure, and dehydrogenation reactions.

Pulcherralpin (**1**) was inactive in the KB and P-388 in vitro test systems (15).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Specific rotation was measured on a Perkin-Elmer 241 polarimeter. The uv spectrum was obtained with a Beckman DU-7 Spectrophotometer, and ir spectrum on a Nicolet MX-1 FT-IR Spectrometer. Nmr spectra were recorded on a Nicolet NMC-360 instrument, operating at 360 MHz for proton resonance and 90.8 MHz for carbon resonance, or a Nicolet NMC-200 (200 MHz) instrument. TMS was used as an internal standard, and chemical shifts were expressed in δ (ppm). Low-resolution ms was obtained with a Finnigan Mass Spectrometer, Model 4500, operating at 70 eV. Cd data were determined in a JASCO J-40A automatic recording spectropolarimeter.

PLANT MATERIAL.—Stems of *C. pulcherrima* were collected in Sri Lanka in 1978. Voucher specimens have been deposited at the Herbarium of the Field Museum of Natural History, Chicago, Illinois; the Kew Herbarium, London, England; and the Conservatoire et Jardin Botanique, Geneva, Switzerland.

EXTRACTION AND FRACTIONATION.—A MeOH extract of the dried stems (32 kg) was concentrated in vacuo to yield 1.9 kg of residue. The residue was then taken up in MeOH-H_2O (1:2) and successively partitioned between petroleum ether, $CHCl_3$, and BuOH.

[1]Fachinformationszentrum, Energie, Physik, Mathematik GmbH, D-7514 Eggenstein-Leopoldshafen 2, West Germany.

CHROMATOGRAPHY OF THE $CHCl_3$ FRACTION.—An aliquot of the $CHCl_3$ fraction (180 g) was chromatographed on a column of silica gel (5 kg) packed in petroleum ether-$CHCl_3$ (17:3). A total of 114 fractions (1 liter each) was collected as the solvent was progressively changed to increasing polar mixtures of petroleum ether/$CHCl_3$, $CHCl_3$, and $CHCl_3$/MeOH. Fractions 42-53 (7.9 g) were rechromatographed on a column of silica gel (250 g) and eluted with progressively increasingly polar mixtures of $CHCl_3$ and EtOAc. Fractions of 20 ml each were collected.

ISOLATION OF PULCHERRALPIN (**1**).—Fractions 71-112 of the second column yielded a white precipitate at room temperature. Repeated crystallization from Me_2CO afforded colorless needles of pulcherralpin (**1**, 325 mg), mp 243-246°; $[\alpha]_D$ +22.5 ($CHCl_3$, *c* 0.27); uv (EtOH) λ max 209 (log ε 4.14), 220 (4.27), 226 (4.23), 280 (4.50); ir (KBr) ν max 3575, 3550, 1735, 1725, 1715, 1635, 1250, 980 cm^{-1}; ^1H nmr (360 MHz), see Table 1; ^{13}C nmr (90.8 MHz), see Table 2; ms *m/z* (rel. int.) 512 (M^+, $C_{30}H_{40}O_7$, 0.2%), 481 (1), 439 (0.5), 364 (2.5), 346 (2), 331 (1), 313 (1), 131 (100); cd (EtOH) Δε (nm) +3.88 (265).

ACETYLATION OF PULCHERRALPIN (**1**).—Compound **1** (10 mg) was treated with Ac_2O in pyridine (0.5 ml each) at 4° overnight. Usual workup afforded a monoacetate **2** (7 mg, gum), whose ^1H-nmr data are shown in Table 1.

HYDROLYSIS OF PULCHERRALPIN (**1**).—Compound **1** (10 mg) was dissolved in a warm solution of 0.1N NaOH in MeOH (4 ml). The alkaline solution (*p*H 11) was left at room temperature overnight and neutralized with dilute HCl. After removal of the MeOH in vacuo, the residue was redissolved in $CHCl_3$ and washed with H_2O (10 ml each). The aqueous portion was extracted twice more with equal volumes of $CHCl_3$. The combined $CHCl_3$ layers afforded a crystalline compound **3** (4 mg) from Et_2O, mp 225-228°; ^1H-nmr (200 MHz), see Table 1; ms *m/z* (rel. int.) 382 (M^+, $C_{21}H_{34}O_6$, 6%), 364 (2), 351 (8), 309 (8), 280 (10), 265 (7), 247 (21), 211 (18), 179 (13), 109 (36), 82 (100).

ACKNOWLEDGMENTS

This investigation was supported, in part, by a grant (CA 33047) from the Division of Cancer Treatment, National Institutes of Health, Bethesda, Maryland. The plant materials used in this study were obtained from 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 77918 C). One of us (DDM) was the recipient of a Predoctoral Fellowship awarded by the National Institutes of Health, 1982-83. The assistance of Dr. S. Balasubramanian, Department of Botany, University of Peradeniya, Sri Lanka, and Dr. D.D. Soejarto, University of Illinois, during the botanical field work is sincerely appreciated. We also thank Ms. S. Yeh and L.J. Lin, University of Illinois, for obtaining the nmr spectra, and the Clinical Pharmacokinetic Laboratory, University of Illinois College of Pharmacy, for providing the mass spectra. We 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. Finally, Drs. Clemens A.J. Erdelmeier and Gian-Andrea Gross, of the Pharmazeutisches Institut, Eidgenossische Technische Hochschule Zurich, are thanked for the INKA Computer-generated carbon spectrum.

LITERATURE CITED

1. D.D. McPherson, G.A. Cordell, D.D. Soejarto, J.M. Pezzuto, and H.H.S. Fong. *Phytochemistry*, **22**, 2835 (1983).
2. D.D. McPherson, C.T. Che, G.A. Cordell, D.D. Soejarto, J.M. Pezzuto, and H.H.S. Fong, *Phytochemistry*, **25**, 167 (1986).
3. E. Quisumbing, "Medicinal Plants of the Philippines," Manila Bureau of Printing, Manila, 1951, pp. 372-374.
4. T.K. Devon and A.I. Scott, "Handbook of Naturally Occurring Compounds," Vol. 2, Academic Press, New York, 1972, p. 187.
5. I. Wahlberg, S.O. Almquist, T. Nishida, and C.R. Enzell, *Acta Chem. Scand.* **29B**, 1047 (1975).
6. H. Eggert, C.L. Van Antwerp, N.S. Bhacca and C. Djerassi, *J. Org. Chem.*, **41**, 71 (1976).
7. J.D. Connolly, F. Orsini, F. Pelizzoni, and G. Ricca, *Org. Magn. Res.*, **17**, 163 (1981).
8. A. Balmain, K. Bjamer, J.D. Connolly, and G. Ferguson, *Tetrahedron Lett.*, **49**, 5027 (1967).
9. A. Balmain, J.D. Connolly, M. Ferrari, E.L. Ghisalberti, N.M. Pagnoni, and F. Pelizzoni, *Chem. Commun.*, 1244 (1970).
10. P. Sengupta, S. Roy and K.G. Das, *Chem. Ind. (London)*, 534 (1970).
11. K.K. Purushothaman, K. Kalyani, K. Subramian, and S. Shanmuganathan, *Indian J. Chem.*, **20B**, 625 (1981).
12. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).

13. W. Moffitt, R.B. Woodward, A. Moscowitz, W. Klyne, and C. Djesassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961).
14. W. Herz and J.J. Schmid, *J. Org. Chem.*, **34**, 2775 (1969).
15. R.I. Geran, N.H. Greenberg, M.M. McDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother Rep. Part 3*, **2**, 1 (1972).

Received 6 August 1985