Journal of Natural Products
Vol. 46, No. 5, pp. 671-674, Sep-Oct 1983

TRADITIONAL MEDICINAL PLANTS OF THAILAND. IV. 3-(2′,3′-DIACETOXY-2′-METHYL BUTYRYL)-CUAUHTEMONE FROM *PLUCHEA INDICA*[1]

SIBABRATA MUKHOPADHYAY,[2] GEOFFREY A. CORDELL,*

Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612

NIJSIRI RUANGRUNGSI, SUPREEYA RODKIRD, PAYOM TANTIVATANA

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 5, Thailand

and P.J. HYLANDS

Pharmacognosy Research Laboratories, Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3 6LX, UK

ABSTRACT.—The structure of 3-(2′,3′-diacetoxy-2′-methyl butyryl)-cuauhtemone (**1**), a new eudesmane derivative from the leaves of *Pluchea indica* (Compositae) has been established through high field proton nmr spectroscopy and chemical correlation.

The genus *Pluchea* in the Compositae, tribe Inuleae, is composed of 50 species distributed in the New World and Far East. A number of *Pluchea* species are noted for their ethnomedical properties, of which the reputed emmenagogue (2-4) and abortifacient (5,6) activities of *Pluchea odorata* in the region of Central America and Caribbean are probably the best known. Extracts of *Pluchea lanceolata* have shown uterine relaxation activity at low doses (7) and possibly both anti-implantation and abortifacient effects (8).

Pluchea indica Less. (syn. P. *foliosa* D.C., *Coryza corymbosa* Roxb., *C. indica* Miq., *Baccharis indica* L.), also known as kukronda, has no established *in vitro* or *in vivo* activities, although in Thailand and Java the leaves and root have been reported to possess astringent and antipyretic properties and are used as a diaphoretic in fevers. Fresh leaves are used in the form of poultices against atonic and gangrenous ulcers (9). Cigarettes prepared from the chopped stem bark are smoked to relieve the pain of sinusitis (10), and in Indo-China, the leaves and young shoots are crushed, mixed with alcohol, and applied to the back in cases of lumbago and also are used for rheumatic pains and in baths to treat scabies (11).

A number of compounds have been isolated from *Pluchea* species, the most characteristic of which are the eudesmane derivatives in the cuauhtemone series from *P. chingoyo* (12), *P. foetida* (13), *P. odorata* (14,15), *P. rosea* (16), and *P. suaveolens* (17). There have been no previous reports of any phytochemical studies on *P. indica*.

In this report we describe the isolation of a new cuauhtemone derivative **1** from *P. indica* and elucidate its structure through interpretation of the high-field proton nmr spectrum and hydrolysis to cuauhtemone (**2**).

Mass measurement of the molecular ion of the isolate established a molecular formula $C_{24}H_{36}O_8$, and of particular interest in the mass spectrum were the ions at *m/z* 217 ($C_{15}H_{21}O$) and *m/z* 131 ($C_6H_{11}O_3$). Whereas the latter suggested a polyfunctional ester unit, the former indicated the nucleus to be a sesquiterpene. Based on prior phytochemical work with *Pluchea* species it was considered that the nucleus might be of

[1]For the previous paper in this series see reference (1).

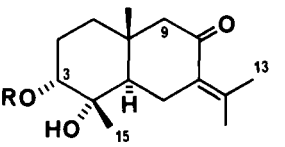
[2]Present address: Regional Research Laboratory, C.S.I.R., Jammu, Tawi, India.

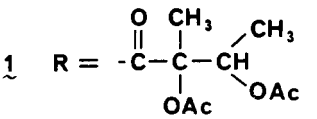
the cuauhtemone type. From the ir spectrum, hydroxy, saturated ester, and α,β-unsaturated ketone functionalities were evident. From the λ max at 258 nm it was apparent that the ketone was α,β,β-trisubstituted (calcd 254 nm), and this was confirmed by the absence of any olefinic proton below 5.4 ppm.

The structure of the isolate was established by the complete assignment of the 400 MHz proton nmr spectrum through use of double resonance and INDOR techniques. Preliminary examination of the spectrum in comparison with published nmr spectral data for compounds in this series (13,14,17), indicated the presence of two acetates at δ 2.09, quaternary methyl groups at C-10 (0.98 ppm) and C-4 (1.28 ppm), and two olefinic methyl groups at δ 1.86 (13-H_3) and δ 2.10 (12-H_3). Two other three-proton singlets in the spectrum (δ 1.26 and 1.66) were assigned to groups in an ester side chain. These data suggested a cuauhtemone derivative esterified with a side chain having the molecular formula $C_9H_{13}O_5$. The presence of a downfield quartet at δ 5.24 coupled to the three-proton doublet (J=6 Hz) at δ 1.26 was confirmed through irradiation and indicated that this must be a 2,3-diacetoxy-2-methyl butyrate unit. It, therefore, remained for us to establish the location of the acylating unit.

Mild hydrolysis of the isolate afforded cuauhtemone (**2**) thereby establishing the skeleton and the location and stereochemistry of the hydroxy groups. This stereochemical array was confirmed through irradiation of the triplet (J=3 Hz) at δ 5.02, which considerably simplified the complex multiplet in the region δ 1.84-1.76. Irradiation at δ 1.81 confirmed the coupling to an equatorial 3β-H and also permitted assignment of the 1α-H to a slightly broadened doublet (J=15 Hz) at δ 1.49 and the 1β-H to a doublet at δ 1.33 in the decoupled spectrum.

Two aliphatic doublets (J=15 Hz) at δ 2.17 and 2.25 were assigned to the 9-H_2. Irradiation at δ 2.17 collapsed the signal at δ 2.25 to a singlet, and this coupling was further substantiated through INDOR experiments monitoring each proton successively. Individual assignment of the 9α-H and 9β-H resonances was based initially on prior data (13,14,17) for compounds in this series.





1

2 R = H

The slightly broadened doublet of doublets (J=4, 13 Hz) at δ 1.92 was assigned to the 5α-H, and irradiation collapsed the signal at δ 3.01 to a doublet (J=15 Hz), which must therefore be the 6α-H. Although significant changes were also observed in the region δ 2.15-2.27, it was only through irradiation at δ 2.17 that the signals at δ 3.01 and 1.92 were collapsed to doublets (J=4 Hz) permitting the assignment of the 6β-H. Only the methylene protons at C-2, both of which must be in the region δ 1.76-1.86 remained to be assigned. Because irradiation at δ 1.49 (1α-H) simplified the region around δ 1.84 more than irradiation at δ 1.33, the 2β-H must be in this region and the 2α-H at about δ 1.81. Because of the non-first-order nature of this part of the spectrum, these values must be considered approximate.

That the ester group is at C-3 and the hydroxy group is at C-4α (rather than in the side chain) is apparent from the 3β-H at δ 5.02 and the 4-CH_3 at δ 1.28. These data are in agreement with those for a 4-hydroxy derivative and not for the corresponding 4-acetyl derivative, where these signals are observed at δ 5.86 and 1.60, respectively (14). The isolate, therefore, has the structure 3-(2′,3′-diacetoxy-2′-methyl butyryl)-cuauhtemone (**1**).

One further interesting observation was made; namely, that the 1α-, 5α- and 9α-resonances were all slightly broadened. This was particularly clear for the 1- and 9-methylene protons where direct comparison could be made with the corresponding β-protons, which were invariably sharp. We suggest this broadening to be caused by weak coupling with the 10-methyl group. Indeed, the resolution-enhanced, 400-MHz spectrum clearly shows this methyl group to be broadened significantly in comparison with the 5′-H_3.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. The uv spectra were measured on a Beckman model DB-G grating spectrometer and the ir spectra were obtained on a Nicolet MX-1 Fourier Transform interferometer with calibration at 1601 cm^{-1}. Proton nmr spectra were recorded at 60 MHz on a Varian Model T-60A instrument, equipped with a Nicolet model TT-7 Fourier Transform attachment and at 400 MHz using a Brüker WH 400 instrument. Tetramethylsilane was used as an internal standard and chemical shifts are reported on the ppm scale. Low resolution mass spectra were obtained with a Varian Mat 112S double-focusing spectrometer operating at 70 eV. The high resolution mass spectrum was obtained with an AEI MS 902 instrument.

PLANT MATERIAL.—The leaves of *Pluchea indica* Less. (Compositae) used in this study were obtained from Nakornpathom, Thailand, during April-May, 1981. The plant materials were authenticated by comparison with voucher specimens at the Botanical Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand

EXTRACTION.—The powdered fresh leaves of *P. indica* (50 kg) were macerated twice, for two-day periods, with 95% ethanol (70 and 50 liters). After combination, the extracts were evaporated *in vacuo*, and the residue was suspended in warm 10% ethanol (10 liters) and filtered. The filtrate was treated with a 5% aqueous lead acetate solution until no further precipitation occurred. The final solution was extracted with chloroform (8 x 500 ml), and the combined chloroform extracts were dried over Na_2SO_4, and evaporated *in vacuo* to afford a yellow, syrupy mass (5.5 g).

ISOLATION OF 3-(2′,3′-DIACETOXY-2′-METYL BUTYRYL)-CUAUHTEMONE (**1**).—The chloroform-soluble fraction (5.5 g) was divided into 11 portions, and each portion was dissolved in chloroform (2 ml), mixed with a small amount of silica gel, and dried. The residue was placed on a dry, silica gel column (2.5 x 40 cm) and eluted with ether to obtain 15-ml fractions. Fractions 11-14 were homogeneous by tlc and afforded colorless prisms (110 mg) of **1** on standing, mp 165°, ir, ν max (KBr) 3420, 2960, 2940, 2890, 1740, 1660, 1585, 1445, 1390, 1370, 1240, 1200, 1120, 1070, 1020, 940, 870, 750, and 620 cm^{-1}; uv, λ max (EtOH) 258 nm (log ε 4.15); ^{1}H-nmr, (400 MHz, $CDCl_3$) δ 0.98 (3H, bd, s, 14-H_3), 1.26 (3H, d, J=6 Hz, 4′-H_3), 1.28 (3H, s, 15-H_3), 1.33 (1H, dt, J=3, 3, 15 Hz, 1β-H), 1.49 (1H, bd, ddd, J=3, 12, 15 Hz, 1α-H), 1.66 (3H, s, 5′-H_3), 1.81 (1H, m, 2α-H), 1.84 (1H, m, 2β-H), 1.86 (3H, s, 13-H_3), 1.92 (1H, dd, J=4, 13 Hz, 5α-H), 2.09 (6H, s, 2 x OAc), 2.10 (3H, s, 12-H_3), 2.17 (1H, d, J=15 Hz, 9α-H), 2.17 (1H, dd, J=13, 15 Hz, 6β-H), 2.25 (1H, d, J=15 Hz, 9β-H), 3.01 (1H, dd, J=4, 15 Hz, 6α-H), 5.02 (1H, t, J=3 Hz, 3β-H) and 5.24 (1H, q, J=6 Hz, 3′-H); ms, *m/z* (rel. int., %) 452 (M^+, 1), 434 (2), 218 (8), 217 (47), 216 (26), 201 (11), 193 (5), 173 (5), 159 (8), 149 (6), 131 (22), 123 (9), 121 (8), 109 (8), 97 (10), 95 (15), 83 (12), 81 (13), 71 (16), 69 (19), 67 (12), 56 (27) and 55 (25). Mass measurements, Obsvd. 452.2412, $C_{24}H_{36}O_8$ requires 452.2410; 217.1594, $C_{15}H_{21}O$ requires 217.1591; 131.0704, $C_6H_{11}O_3$ requires 131.0707.

HYDROLYSIS OF **1**.—To a solution of **1** (11.4 mg) in anhydrous MeOH (10 ml) was added a small quantity of Na_2CO_3. After 2 h at room temperature, the solvent was removed *in vacuo*, cold H_2O (10 ml) was added and the mixture extracted with $CHCl_3$ (5 x 10 ml), and with H_2O until neutral, dried over Na_2SO_4 and evaporated *in vacuo* to afford a gummy mass (**2**, 4.4 mg), uv, λ max (EtOH) 258 nm (4.03); ^{1}H-nmr (60 MHz, $CDCl_3$), δ 0.93 (3H, s, 15-H_3), 1.21 (3H, s, 14-H_3), 1.81 (3H, s, 13-H_3), 2.01 (3H, s, 12-H_3), 2.19 (2H, m, 9-H_2), 2.67 (1H, br s, exchangeable with D_2O, 3-OH), 2.88 (1H, br s, ex-

changeable with D_2O, 4-OH), 3.01 (1H, m, 6α-H) and 3.65 (1H, br s, 3-H); ms, *m/z* (rel. int., %) 252 (M^+, 6), 201 (11), 194 (20), 178 (6), 177 (4), 175 (4), 166 (6), 165 (6), 152 (17), 151 (11), 149 (9), 137 (9), 125 (21), 124 (15), 123 (11), 121 (11), 110 (13), 109 (14), 95 (11), 84 (14), 83 (14), 81 (12) and 73 (64). These physical data are in agreement with those obtained previously for cuauhtemone (**2**) (14), and identity was confirmed by comparison (tlc, ms) with an authentic sample.

ACKNOWLEDGMENTS

The authors would like to thank Dr. M.C. Wani, Research Triangle Institute, NC, for the high solution mass spectrum; Dr. G. Hawkes, Queen Mary College, University of London, for the 400 MHz ^{1}H-nmr spectra; and Professor K. Nakanishi, Department of Chemistry, Columbia University, New York, NY, for an authentic sample of cuauhtemone.

LITERATURE CITED

1. N. Ruangrungsi, P. Tantivatana, R.P. Borris, and G.A. Cordell, *J. Sci. Soc. Thailand*, **7**, 123 (1981).
2. N. Souza-Novelo, Farmacopea Maya, Imprenta "Gamboa Guzman," Merida, Yucatan, Mexico, 1940.
3. I. Rivera, *An. Inst. Biol. Mex.*, **14**, 38 (1943).
4. J. Eerhault, Flore Illustrée de Sénegal II. Govt. Senegal, Ministry of Rural Development, Water and Forest Division, Dakar, 1974.
5. E.S. Ayensu, Medicinal Plants of the West Indies, Unpublished manuscript.
6. M. Steggerda and B. Korsch, *Bull. Hist. Med.*, **13**, 54 (1943).
7. D.N. Prasad, K.D. Gode, P.S. Sinha, and P.K. Das, *Indian J. Med. Res.*, **53**, 1062 (1965).
8. Central Drug Research Institute, Annual Report, Lucknow, India, p. 1, 1978.
9. "The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Vol. VIII." C.S.I.R., Delhi, India, 1948.
10. S. Pongboonrod, "Maitet Meang Thai," Kasembunakij Press, Bangkok, 1971, p. 117.
11. L.M. Perry, "Medicinal Plants of East and Southeast Asia," MIT Press, Cambridge, MA, p. 96, 1980.
12. M.T. Chiang, M. Bittner, M. Silva, W.H. Watson, and P.G. Sammes, *Phytochemistry*, **18**, 2033 (1979).
13. F. Bohlmann and P.K. Mahanta, *Phytochemistry*, **17**, 1189 (1978).
14. K. Nakanishi, R. Crouch, I. Miura, X.A. Dominguez, A. Zamudio, and R. Villarreal, *J. Am. Chem. Soc.*, **96**, 609 (1974).
15. F. Bohlmann and C. Zdero, *Chem. Ber.*, **109**, 2653 (1976).
16. X.A. Dominguez, R. Franco, G. Gano, R. Villarreal, M. Bapuji, and F. Bohlmann, *Phytochemistry*, **20**, 2297 (1981).
17. F. Bohlmann, J. Ziesche, R.M. King, and H. Robinson, *Phytochemistry*, **19**, 969 (1980).

Received 28 October 1982