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NEW MACROCYCLIC Δ^1-PIPERIDEINE ALKALOIDS FROM PAPAYA LEAVES: DEHYDROCARPAINE I AND II*

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Key Word Index—*Carica papaya*; Caricaceae; piperideine alkaloids; piperidine alkaloid; carpaine; dehydrocarpaines.

Abstract—Two new Δ^1-piperideine alkaloids, dehydrocarpaine I and II, have been isolated from papaya leaves by TLC and their structures elucidated by MS and PMR. Both alkaloids have higher concentrations than that of carpaine, or the only major alkaloid previously reported in this plant.

INTRODUCTION

The alkaloid carpaine is a dimeric, macrocyclic lactone with two piperidine units incorporated into the ring. First isolated in 1890 from papaya (*Carica papaya* L.) leaves by Greshoff [1], the determination of the structure of carpaine was not completed until 1965 [2]. More recently, a new method was developed for the synthesis of carpaine and several other macrocyclic lactones, which represents an increasingly important class of biologically active molecules [3].

Carpaine has been reported to possess varieties of pharmacological activities [4], and it was believed to be the major alkaloid in papaya leaves [5]. A stereoisomer, pseudocarpaine, was later found in small quantity by Govindachari *et al.* [6]. Other naturally occurring piperidine alkaloids with similar structures to that of carpaine have been reported in *Azima tetracantha* Lam. (Salvadoraceae) [7]. In addition to carpaine which consists of a 26-membered ring, this plant also has azimine and azicarpaine, the corresponding 22- and 24-membered ring analogues of carpaine. The present report describes the identification of two new major alkaloids, dehydrocarpaine I and dehydrocarpaine II, in papaya leaves.

RESULTS AND DISCUSSION

Two grams of crystalline carpaine were obtained from 5 kg of dried leaves according to the procedure of Coke and Rice [2]. The crystals had a mp of 119–120° (lit. 119–120°); the mass and PMR spectra were in agreement with the reported data [6, 7]. TLC of the crude alkaloid extract however, showed 3 Dragendorff positive spots. Carpaine at R_f 0.4 appeared as the least prominent of the 3. Isolation and purification of the unknowns were achieved by TLC using 3 solvent systems. Both compounds appear as viscous oils. The MS of the R_f 0.24 fraction showed a M^+ at *m/e* 476, 2 amu less than that of carpaine. The higher fragments at *m/e* 461, 434, 405, 382 and 333 were also 2 amu less than those of the corresponding carpaine fragments [6, 8], indicating that it is a carpaine analogue with one additional double bond. The MS of the R_f 0.15 fraction showed a M^+ at *m/e* 474, and two major fragments at 459 and 331, suggesting a tetradehydro analogue of carpaine. Catalytic hydrogenation over Pd–C[3] transforms the unsaturated analogues to carpaine, which was confirmed by both R_f values and MS.

The R_f values of carpaine, dehydrocarpaine I and dehydrocarpaine II are 0.40, 0.24 and 0.15 in solvent system I, respectively. In solvent system II, however, carpaine has the lowest R_f value of 0.12, next to dehydrocarpaine I (0.33) and dehydrocarpaine II (0.51). Reversal of the sequences in the above acidic system I and neutral system II developing solvents suggests that the nature of the N atoms of these 3 compounds are quite different, which leads to the assumption that imino bond(s) would be present in the unknowns.

Evidence from PMR spectra leads to the conclusion that these two unknown compounds are Δ^1- and $\Delta^{1,1'}$-piperideine analogues of carpaine: The two Me groups on carpaine C-2 and C-2′ appeared as a doublet at δ = 1.01 ppm (J = 7 Hz). This doublet was reduced in dehydrocarpaine I, in which only the C-2H was present and, in dehydrocarpaine II, the doublet disappeared entirely indicating the absence of both C-2 and C-2′Hs. The double bond also caused a downfield shift of the singlet CH_3 and the signal was integrated into the multiplet of methylene protons around 1.3 ppm. The broad quartet at δ 2.85 (C-2 and C-2′ Hs) as well as the amino-H at 4.74 ppm also responded accordingly. The new broad triplet that appeared at δ = 4.02 ppm in the dehydrocarpaines is assigned to the H on C-3 and C-3′. This proton would become recognizable after the limination of the proton on C-2 and/or C-2′.

We have examined several inbred lines of *C. papaya* L. common in Hawaii, all of them showed that carpaine is in fact the least prominent analogue among the 3. While failure of recognizing these two major alakloids in the past can be attributed to the fact that they do not crystallize readily as carpaine does, it is interesting to note that the yield of carpaine from papaya leaves varied widely [9]. In this regard, it is suggested that differences in the proportion of the 3 analogues in different papaya varieties be taken into consideration, together with other possible factors.

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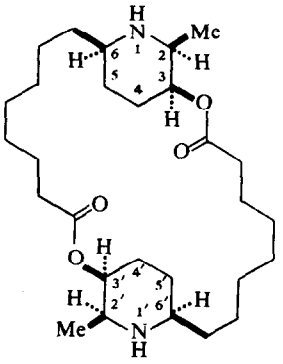
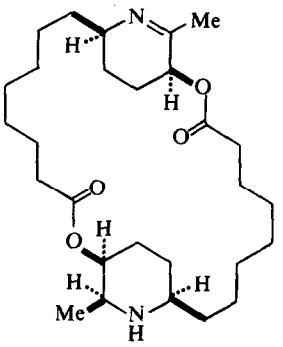
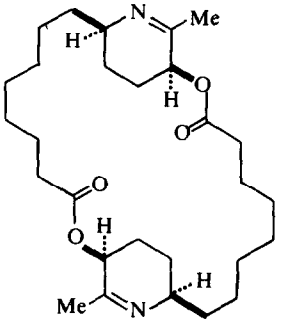


Carpaine



Dehydrocarpaine I



Dehydrocarpaine II

Biosynthesis of acetate-derived piperidine alkaloids has been studied most extensively with coniine in hemlock (*Conium maculatum* L.). It is now well established [10] that coniine is formed by the 'polyketide' pathway through a intermediary Δ^1 piperidine, γ-coniceine. As for carpaine, Bevan and Ogan [11] reported that ^{14}C-labelled acetate served as the most efficient precursor and suggested that carpaine also originated from the 'polyketide' route. The present finding of the two Δ^1-piperideines as the major papaya alkaloids further raises the possibility that, similar to γ-coniceine in coniine biosynthesis, the two dehydrocarpaines are intermediary metabolites in the carpaine biosynthetic pathway.

EXPERIMENTAL

Carpaine was prepared by the procedure of ref. [2]. Papaya (*Carica papaya* L. Solo, Line 77) leaves were collected from the Waimanalo Experimental Farm, University of Hawaii. A total of 5 kg of air-dried leaves were powdered and soaked in 6 l. of 89% EtOH, 10% H_2O and 1% HOAc for 2 days. The extract was decanted and the extraction repeated. The combined extracts were evaporated at *ca* 60° in a rotary evaporator to a thick syrup which was shaken with 1 l. of H_2O containing 20 ml HOAc. The mixture was extracted with Et_2O and the aq. phase was separated, adjusted to pH 11 with K_2CO_3 and then extracted thoroughly with Et_2O. The Et_2O extract was washed with H_2O, extracted with 5% HCl and the acid extract was again adjusted to pH 11 and extracted with Et_2O. This Et_2O soln, after drying over dry $MgSO_4$, was concd to a dark brown 'crude extract' of papaya leaf alkaloids, from which carpaine precipitated on chilling. *Ca* 2 g of twice recrystallized carpaine was collected.

Isolation of dehydrocarpaines. The 'crude extract' described above was analysed by prep-TLC on Si Gel G in solvent system I (BuOH–HOAc–H_2O; 4:1:5). Three Dragendroff positive bands were collected and extracted with $CHCl_3$. The R_f 0.4 band was carpaine, the R_f 0.24 and 0.15 fractions were rechromatographed in solvent systems II (MeOH–$CHCl_3$; 3:7) and III ($CHCl_3$–C_6H_6; 1:9), consecutively. *Ca* 2 mg each of viscous oils were obtained from these two bands.

Catalytic hydrogenation. A small portion of the 'crude extract' was dried under N_2 in a 5 ml round-bottom flask and the residue (*ca* 2 mg) was dissolved in 1.5 ml of EtOH. 5 mg of Pd–C (5%, Matheson, Coleman and Bell) and 1 μl of conc HCl were added and a gentle stream of H_2 was passed into the mixture at room temp. [3]. An ice-cold condenser was used to prevent solvent lost. Microliter quantities of samples were removed from the reaction bottle for TLC analyses during the course of the catalytic hydrogenation.

MS and PMR. High resolution MS was performed at 70 eV on a double focusing instrument. For general examination of the isolated alkaloids, however, a quadrupole low resolution spectrometer was routinely used. PMR (100 MHz) were measured in $CDCl_3$ with TMS as internal standard.

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