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SHORT COMMUNICATION

FORMATION OF DIACETONAMINE AND TRIACETONAMINE IN PLANT EXTRACTS*

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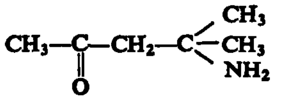
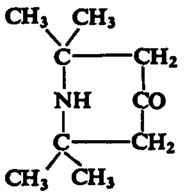
Abstract—Diacetonamine and triacetonamine were isolated from extracts of tangerine leaves and shown to be artifacts formed during the isolation procedure.

DURING an investigation of nitrogenous constituents in tangerine leaves, two apparently new compounds were isolated. The compounds were identified by elemental and i.r. analyses and found to be 2-amino-2-methyl-4-pentanone (diacetonamine) (I) and 2,2,6,6,-tetramethyl-4-piperidone (triacetonamine) (II). It is believed that these compounds are artifacts formed during the extraction from acetone and ammonia.



$CH_3-\underset{\underset{O}{\|}}{C}-CH_2-C\begin{smallmatrix}CH_3\\CH_3\\NH_2\end{smallmatrix}$

(I)



(II)

EXPERIMENTAL

Finely ground, dried tangerine leaves were extracted with methanol and approximately 50 gallons of the extract were passed through an ion exchange resin column (Dowex-50W X 8, 200–400 mesh in the ammonium form). The resin was washed with acetone, water, and finally with methanol. The basic nitrogenous constituents were then eluted with 2 N NH_4OH in methanol. The eluate was concentrated to a small volume in a rotary vacuum evaporator at 30° and then neutralized with dry oxalic acid. In addition to the formation of synephrine oxalate, large amounts of other crystals were formed. The latter were separated by dissolving them in methanol. A microscopic examination of the methanol-soluble crystals showed the presence of both needles and thin plates suggesting a mixture of at least two compounds. The mixture was decolorized with Darco G-60 activated carbon and recrystalized four times from hot 95% ethanol. Some of the crystals were dissolved in water which was made strongly basic with 10% NaOH, and the triacetonamine (II) was separated by extracting with ethyl ether. The remaining water solution was then extracted with chloroform which removed the diacetonamine (I). Dry sodium sulfate was added to the ether and chloroform fractions to remove the excess water. Each was then taken to a small volume, diluted with 95% ethanol and neutralized with oxalic acid.

The diacetonamine free base was regenerated from the oxalate salt by adjusting a water solution of the salt to pH 8·4 with NH_4OH and passing it through an ion exchange resin column (Dowex-50W X 8, 200–400

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mesh in the ammonium form) and eluting with NH_4OH in methanol. The excess ammonia was removed and the HCl salt was formed by neutralization with anhydrous HCl in methanol. (Found: C, 47·37; H, 9·38; N, 9·38; O, 10·58: Calc. for $C_6H_{13}NO.HCl$:C, 47·52; H, 8·63; N, 9·23; O, 10·55%.) A commercial source of diacetonamine was found to be impure and not useful in identifying the unknown. Diacetonamine was then synthesized from mesityl oxide and ammonia.[1] The oxalate of the synthesized compound and that taken from the plant extract gave identical i.r. absorption curves.

The triacetonamine was identified by elemental analyses of the picrate. (Found: C, 46·84; H, 5·35; N, 14·86; O, 33·02 Calc. for $C_{15}H_{20}O_8N_4$:C, 46·86; H, 5·25; N, 14·59; O, 33·33%.) I.r. absorption curves of the free base, oxalate, and picrate of commercially available triacetonamine and the compound isolated from the leaf extract were identical.

An attempt was made to determine if diacetonamine and triacetonamine were artifacts formed during the extraction process. Tangerine leaves were extracted as described except that 10% ethyl ether in methanol was used instead of acetone in washing the column. When the free amines were then eluted, there was no indication of either diacetonamine or triacetonamine. In a further test on the formation of these ketoamines, two 400-ml aliquots of the eluate taken from the ether washed resin were put into flasks. To one was added approximately 100 ml of acetone. The two flasks were then corked and placed on a laboratory shelf for about 2 months. The contents of each flask was reduced to a small volume in a rotary vacuum evaporator at 30°, diluted with 95% ethanol, and neutralized with oxalic acid. The extract to which acetone had been added contained large amounts of both diacetonamine and triacetonamine. Neither of these compounds were detectable in the extract to which no acetone had been added.

Triacetonamine has been reported in the plant *Acalypha indica* in South Africa.[2] The authors did say, however, that they were unable to isolate this compound when an alternate method of extraction was attempted. Bierman[3] describes the identification of diacetonamine from plant material and concluded that it was an artifact. He gave no details about the extraction method used. Housholder and Camp[4] recently pointed out that alkaloid artifacts were readily formed during extractions using ammonium hydroxide and acetone. These workers, however, did not identify any of the artifacts.

We believe the potential problems associated with the use of acetone in preparation of biological materials is frequently overlooked. Due to the reactions with ammonia or with naturally occurring compounds, acetone should be used with caution.

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[2] C. RIMINGTON and G. C. S. ROETS, *Onderstepoort J. Vet. Sci. Anim. Ind.* **9**, 193 (1937).

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[4] D. E. HOUSHOLDER and B. J. CAMP, *J. Pharm. Sci.* **54**, 1676 (1965).