[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE RICE INSTITUTE]

The Mustard Oil of Papaya Seed

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Received October 10, 1955

Papaya seed macerated in water or an organic solvent afforded no thioureas, but 0.2–0.5% of benzyl isothiocyanate.

Guignard[1] showed over sixty years ago that the seed of papaya (*Carica papaya* L.) appeared to furnish an isothiocyanate, liberated enzymatically on maceration and tasting like garden cress[2] or nasturtium, which are now known to contain benzyl isothiocyanate. He established that the seed coat or embryo could decompose the glucoside sinigrin to allyl isothiocyanate, and that the papaya mustard oil could be formed from the seed albumen by action of an extract of white mustard seed, containing the enzyme myrosin. Much later, Panse and Paranjpe[3] reported the isolation of benzylthiourea ("carpasemine") in 0.35% yield from dry papaya seed. No other derivative of thiourea, save the 2-thioimidazolone ergothioneine, is known to occur naturally. It has been remarked[4] that since Panse and Paranjpe extracted the seed with an ammoniacal solvent, Prollius' fluid,[5] the thiourea obtained might have been an artifact formed from benzyl mustard oil. We present experimental evidence that the primary substance is indeed benzyl isothiocyanate.

Mexican papaya seed was analyzed for thioureas by maceration in water, extraction with ether, and measurement of ultraviolet absorption. The content of benzylthiourea was undetectably small, less than 0.03%. The seed did afford some 0.23% by assay of benzyl isothiocyanate, isolated in amount of 0.14%. The substance was synthesized from benzylamine by decomposition of the carboethoxy dithiocarbamate with base[6] in 80% yield, a substantial increase over the 9% yield reported[7] from uncatalyzed decomposition. The natural and synthetic mustard oils were characterized and identified by infrared spectra and by the derived benzylthiourea and N-benzyl-N′-phenylthiourea.

The only feature of Panse and Paranjpe's work that evokes further comment is that the mustard oil was liberated in a mixture of ether, chloroform, alcohol, and ammonia, which might not ordinarily be considered favorable for enzymatic action. However, others[8] have found that isothiocyanates can be produced from ground seeds in an external medium of organic solvents. The enzyme, myrosin, is reported to exhibit activity in 5% aqueous borax and hence is insensitive to weak base. In fact, spectrophotometric examination of extracts of papaya seed with Prollius' fluid showed the presence of a thiourea, whereas extracts with a similar mixture in which water replaced ammonia contained the isothiocyanate. The process was remarkably efficient, for the yields of benzyl isothiocyanate from seed, judged spectrophotometrically, were 0.5%, greater than those obtained by aqueous maceration. When the crude thiourea was chromatographed,[9] only benzylthiourea was detected.

Proof that benzyl isothiocyanate occurs in the papaya family *Caricaceae* extends the known range of a mustard oil widely distributed among plant families. In the mustard family *Cruciferae*, benzyl isothiocyanate is a principal product only of the garden cress *Lepidium sativum*,[8,10] *L. densiflorum* and *virginicum*,[8] and the closely related *Coronopus didymus*,[8,11] but it is found also in the garden nasturtium, *Tropaeolum majus*[8,12,13] (family *Tropaeolaceae*), the toothbrush tree, *Salvadora oleoides*[14] (*Salvadoraceae*), and the horseradish tree, *Moringa oleifera*[15] (*Moringaceae*). The long known[16] activity against fungi and bacteria of benzyl isothiocyanate, which can pass unchanged through the human body, at concentrations of 0.001–0.0002%

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[1] Guignard, *Compt. rend.*, **118**, 545 (1894); *J. de botanique*, **8**, 67 (1894); *cf.* Hooper, *Pharm. J.*, [4] **37**, 369 (1913).

[2] Hence early Spanish settlers in tropical America, the native region of papaya, called the fruit "higo de mastuerzo" or fig of cress [Standley, *Trees and Shrubs of Mexico, Contribs. U. S. Natl. Herbarium*, **23**, 852 (pt. 4, 1924)].

[3] Panse and Paranjpe, *Proc. Indian Acad. Sci.*, **18A**, 140 (1943).

[4] Astwood, Greer, and Ettlinger, *J. Biol. Chem.*, **181**, 121 (1949).

[5] Mitchell, ed., *Allen's Commercial Organic Analysis*, 5th ed., Blakiston, Philadelphia, 1929, Vol. VII, p. 6.

[6] Hodgkins and Ettlinger, *J. Org. Chem.*, **21**, in press (1956); *cf.* Schultz and Barthold, *Arch. Pharm.*, **285**, 267 (1952).

[7] Backer, Mulder, and Froentjes, *Rec. trav. chim.*, **54**, 57 (1935).

[8] Kjaer, Conti, and Larsen, *Acta Chem. Scand.*, **7**, 1276 (1953).

[9] Kjaer and Rubinstein, *Acta Chem. Scand.*, **7**, 528 (1953).

[10] Gadamer, *Arch. Pharm.*, **237**, 507 (1899).

[11] McDowall, Morton, and McDowall, *New Zealand J. Sci. Technol.*, **28A**, 305 (1947); [*Chem. Abstr.*, **41**, 6637 (1947)].

[12] Gadamer, *Arch. Pharm.*, **237**, 111 (1899). For summary of this paper and ref. 10, *cf.* Gadamer, *Ber.*, **32**, 2335 (1899).

[13] Schneider, Clibbens, *et al.*, *Ber.*, **47**, 1248 (1914).

[14] Patel, Iyer, Sudborough, and Watson, *J. Indian Inst. Sci.*, **9A**, 117 (1926); [*Chem. Zentr.*, **98**, **I**, 465 (1927)].

[15] Kurup and Rao, *Indian J. Med. Research*, **42**, 85 (1954); Ettlinger and Lundeen, unpublished.

[16] Beijerinck, *Zentr. Bakteriol. Parasitenk.*, **Abt. II**, **6**, 72 (1900).

has recently aroused interest.[15,17] The most common use of papaya seed in folk medicine appears to be as an anthelmintic.

The investigation was supported by a research grant (A-238) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

EXPERIMENTAL

Assays. Ground dry papaya seed[18] (50 g.) was macerated in 300 cc. of water during an hour at 55°. A 27-g. sample of the mixture was extracted thoroughly with ether,[19] the ether was evaporated, and the residue was taken up in 20 cc. of methanol. The methanol solution, diluted to twenty-five fold, had an ultraviolet absorption maximum at 250 mμ, optical density (1-cm. cell) 0.147. The peak absorptions of the isothiocyanate[20] and monosubstituted thiourea[8] groups in methanol and ethanol lie respectively at 245–250 mμ, log ϵ 3.0 (found in present work for allyl isothiocyanate in ethanol λ_{max} 252 mμ, log ϵ 2.94) and 243 mμ, log ϵ 4.1. Hence the absorption found corresponds to less than 0.3 mg. of benzylthiourea per g. of dry seed, and to 3 mg./g., neglecting extraneous absorption, of benzyl isothiocyanate.

The remainder of the mash was steam-distilled until 300 cc. were collected. The absorption spectrum in methanol of the distillate (λ_{max} 249 mμ) indicated a yield of 4 mg./g. of benzyl isothiocyanate from seed. The distillate was treated with 15 cc. of aqueous ammonia, let stand overnight, and concentrated. The ultraviolet absorption in water (λ_{max} 238 mμ) corresponded closely in pattern to that of benzylthiourea (λ_{max} 238 mμ, ϵ 14100; ϵ at 220, 240, and 260 mμ, 10300, 13800, and 1700) and was equivalent to a yield of isothiocyanate of 2.3 mg./g. The solution (calc'd content of thiourea 0.12 g.) was evaporated *in vacuo* and the residue, 0.13 g., m.p. 161–163°, was crystallized from alcohol to furnish benzylthiourea, m.p. 164–165°.

One gram of seed was milled[21] ten minutes in 85 cc. of Prollius' fluid, let stand 30 hours and filtered, and the solvent was evaporated. The residual oil (0.3 g.) was boiled with ethanol to expel all chloroform and the spectrum (λ_{max} 243 mμ) was measured in ethanol. The apparent yield of benzylthiourea, corrected[8] for extraneous absorption, was 5 mg.

Two grams of seed were milled 15 minutes in 95 cc. of solvent corresponding to Prollius' fluid without ammonia (100 cc. of ether, 32 cc. of chloroform, 10 cc. of ethanol, and 4 cc. of water, freshly shaken) and let stand 30 hours. The spectrum of the extract, obtained as before, had a low maximum at 243 mμ, but the density at 240 mμ corrected as if to separate absorption by benzylthiourea from a linear term was only 8% of the total,[22] and corresponded to less than 0.3 mg./g. of benzylthiourea in the seed. Furthermore, the extract gave no color with Grote's reagent.[9] A similar extract of 1 g. of seed was treated after filtration with 5 cc. of concentrated ammonia and 25 cc. of ethanol and evaporated next day. A paper chromatogram[9] of the sampled product, which contained 6 mg. of the thiourea according to the spectrum, showed a single, intense spot with R_{Ph} 0.93, corresponding to benzylthiourea.

Isolation. One pound of ground papaya seed was digested in 2.8 l. of water at 55° for an hour and steam-distilled until 3 l. were collected. The distillate was saturated with salt and extracted continuously with ether during 48 hours. The ether was dried and evaporated under a Vigreux column, and the residue was fractionated *in vacuo* to give 0.63 g. of benzyl isothiocyanate, b.p. 90–91° at 1.3 mm. (lit.[7,13] b.p. 143° at 20 mm., 124–125° at 12 mm.), n_D^{26} 1.5955.

A 0.1-g. sample of the papaya mustard oil was heated three hours with 0.75 g. of aniline in 3 cc. of boiling benzene, and the product was crystallized from alcohol to give N-benzyl-N′-phenylthiourea, m.p. 152.5–153.5°. Mixtures with phenylthiourea and thiocarbanilide began to melt at 129° and 138° respectively.

Synthesis. N-Benzyl-N′-phenylthiourea, prepared from benzylamine and phenyl isothiocyanate, melted at 154–154.5° (lit.[23] m.p. 153–154°) and did not depress the melting point of the sample derived from papaya seed.

Benzylamine (7.5 g.) was converted[6] in 80% yield by the procedure exemplified in synthesis of allylcarbinyl isothiocyanate[24] to benzyl isothiocyanate, n_D^{29} 1.5980 (lit.[25] n_D^{15} 1.6049). The infrared absorption spectra between 2.5 and 15 μ of the natural and synthetic mustard oils were identical save for tiny extraneous bands of the natural oil at 8.02 and 8.95 μ.

Synthetic benzyl isothiocyanate was treated with aqueous-alcoholic ammonia at 5° for a day, and the product was crystallized from aqueous ethanol to give benzylthiourea, m.p. 165–165.5° (lit.[3,26] m.p. 165°, 162°), undepressed on mixture with the sample derived from papaya.

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(17) Winter and Willeke, *Naturwissenschaften*, **39**, 236 (1952); **40**, 167 (1953); Winter, *Naturwissenschaften*, **41**, 337, 379 (1954); Halbeisen, *Naturwissenschaften*, **41**, 378 (1954); Das, Kurup, and Rao, *Naturwissenschaften*, **41**, 66 (1954); Gopalakrishna, Kurup, and Rao, *Indian J. Med. Research*, **42**, 97 (1954); *et seq.*

(18) The whole seeds, obtained from market fruit, were generously supplied by Dr. Carl Djerassi and Syntex, S. A., Mexico City. The seeds and gelatinous outer coats were dried one day in sun and eight hours at 70°, with reduction in weight from 3.5 to 0.6 kg.

(19) The distribution of benzylthiourea between ether and water favors the ether phase by an approximate ratio of 2.4.

(20) Pestemer and Litschauer, *Monatsh.*, **65**, 239 (1935). The high extinction reported for methoxymethyl isothiocyanate in methanol may be caused by thiourethan formation, accelerated by an electron-attracting substituent [*cf.* Browne and Dyson, *J. Chem. Soc.*, 3285 (1931); 178 (1934)]. See also Kjaer and Conti, *Acta Chem. Scand.*, **8**, 295 (1954).

(21) With a Sargent Centrifugal Wet Mill, Size 1 (E. H. Sargent and Co., Chicago, Ill.).

(22) The corrected density of extracts treated with ammonia was 65–73% of the observed.

(23) Dixon, *J. Chem. Soc.*, **55**, 300 (1889).

(24) Ettlinger and Hodgkins, *J. Am. Chem. Soc.*, **77**, 1831 (1955).

(25) Hawthorne, *J. Chem. Soc.*, **89**, 556 (1906).

(26) Dixon, *J. Chem. Soc.*, **59**, 551 (1891).