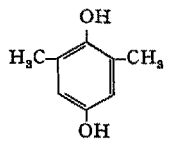
Stabilization of Aqueous Solutions of the Insect Chemosterilant *m*-Xylohydroquinone (*m*-XHQ) by Vitamin C

SANYAL's finding[1] that *m*-xylohydroquinone (*m*-XHQ) is a mammalian oral contraceptive formed the basis of numerous papers released by this author and his co-workers from 1949 until the present time[2]. The anti-fertility effect of *m*-XHQ in the common laboratory rodents is a rather controversial issue[3], since most workers have been unable to confirm it; nevertheless, the compound has been given widespread clinical testing as a contraceptive with Indian women[4,5]. Its relatively low mammalian toxicity[4,6,7] led to its examination as an insect chemosterilant[8–10]. When fed to adult houseflies, we found it to be a rather efficient oviposition-inhibitor for the female[9], and chemosterilant for the male housefly[11,12].



m-Xylohydroquinone (*m*-XHQ)

During these studies a serious drawback of *m*-XHQ as regards its potential use as an insect chemosterilant was noticed, namely, its proneness to oxidation on storage in the solid state and its even much more rapid decomposition (oxidation) in aqueous solutions[11]; e.g. 0.15% aqueous solutions, which are initially colourless, assume within a few minutes a yellow, then a brown-red, and finally a deep wine-red colour. Within a few days the solution then turns through various shades of brown to a final yellow[13]. These colour changes are accompanied by the development of a characteristic smell and the partial to total inactivation of the compound as an insect sterilant[11]. In order to preserve its chemosterilant activity in solutions fed to houseflies, *m*-XHQ had to be dissolved in milk (1 part milk powder in 8 parts water), which stabilizes it[11]. The instability of aqueous solutions prevented the testing and potential application of *m*-XHQ against most insects other than the housefly.

Since oxidation in the alimentary canal seems to be one of the problems encountered with *m*-XHQ as an oral mammalian contraceptive, SANYAL and GHOSH[4] administered the substance with 5%, by weight, of sodium hydrosulphite, the reducing agent they used during the preparation of *m*-XHQ from *m*-xyloquinone. This procedure was also followed by THIERSCH[7]; however, he was not able to obtain with it other than negative biological results in reproduction studies with the female rat. SANYAL very recently (personal communication, 1966) still advised us to employ this adjuvant, but we also found that the addition of 5% the weight of *m*-xylohydroquinone of sodium hydrosulphite to an aqueous solution of the compound had little effect on its decomposition[11]. On the other hand, it was now recognized that dissolving dry *m*-XHQ in aqueous solutions of the antioxidant L-ascorbic acid (vitamin C)[14] stabilizes the compound most effectively, lack of colour of the solution being taken as a first indication that no decomposition had set in. While 0.5% *m*-XHQ in water is effectively protected for a few days by a concentration of as low as 0.05% vitamin C, 0.15% *m*-XHQ, which we fed in our experimental arrangement for 3 days to male houseflies, requires at least 0.2% vitamin C to protect it for such a length of time; 0.3% vitamin C was effective for 4–6 days, while 0.5% gave protection for more than a week.

Vitamin-C-stabilized solutions of *m*-XHQ were thereupon tested biologically, employing a procedure[11] which was, briefly, as follows: 50 freshly emerged housefly

The effect on egg fertility of feeding housefly males for 3 days with different solutions of *m*-xylohydroquinone (*m*-XHQ) before mating them to virgin females

♂♂ fed for 3 days prior to mating	During 14 days after mating		% Average egg fertility	% Average egg fertility, corrected[a]
	Average longevity of ♂♂ (days)	Volume of eggs laid by 50 ♀♀ (ml)		
Milk (control)	11.6	4.0	84.3	
0.2% vitamin C in H_2O	12.8	4.25	84.8	
0.15% *m*-XHQ dissolved in H_2O only	12.8	4.4	81.1	96.2[b]
0.15% *m*-XHQ dissolved in milk	13.1	4.0	2.6	3.1
0.15% *m*-XHQ dissolved in H_2O containing 0.2% vitamin C	12.7	4.1	2.3	2.7

[a] Corrected for control sterility according to a slight transformation of Abbot's formula[11]. [b] Values obtained in other runs for 0.15% *m*-XHQ in water only were 85.0%, 56.6%, 84.5% and 46.6%.

[1] S. N. SANYAL, J. Med. int. med. Abstr. Rev. *19*, 3 (1956).

[2] Over 50 articles published nearly exclusively in Indian journals, mainly in: Sci. Cult.; Calcutta med. J.; Int. med. Abstr. Rev.; J. Med. int. med. Abstr. Rev.; Med. Int.; J. Indian chem. Soc. See for summaries, e.g. S. N. SANYAL et al., Acta endocr., Copenh. Suppl. *28*, 72 et seq. (1956 – four papers); S. N. SANYAL, Pharmaceutist *12*, 21 (1966).

[3] Reviewed in part by H. JACKSON, Pharmac. Rev. *11*, 135 (1959); cf. also U. K. BANIK and H. S. CHAKRAVARTI, Ann. Biochem. exp. Med. *17*, 63 (1957); I. RINGLER and A. KLIMAN, Endocrinology *63*, 135 (1958); J. R. PRICE, in *Agents affecting fertility* (Eds. C. R. AUSTIN and J. S. PERRY; J. & A. Churchill Ltd., London 1965), p. 3.

[4] S. N. SANYAL and S. GHOSH, Acta endocr., Copenh. Suppl. *28*, 83 (1956).

[5] S. N. SANYAL, Med. Int. *27*, 169 (1963).

[6] S. N. SANYAL, S. C. BANERJEE and J. SEN, Acta endocr., Copenh. Suppl. *28*, 93 (1956); H. A. FREYRE, M. ROVIRA and M. I. ARDAO, Archos Soc. Biol. Montev. *24*, 82 (1958); Chem. Abstr. *56*, 4051d (1962).

[7] J. B. THIERSCH, Acta endocr., Copenh. Suppl. *28*, 46 (1956).

[8] M. N. MUKHERJEE, Sci. Cult. *27*, 497 (1961).

[9] K. R. S. ASCHER and I. HIRSCH, Entomologia exp. appl. *6*, 337 (1963).

[10] K. R. S. ASCHER, Int. Pest Control *7* (1), 8 (1965).

[11] K. R. S. ASCHER and N. AVDAT, Int. Pest Control *8* (6), 16 (1966).

[12] K. R. S. ASCHER and N. AVDAT, Int. Pest Control *9* (2), 8 (1967).

[13] It is assumed that this is the final oxidation product 2,6-dimethyl benzoquinone (*m*-xyloquinone) – according to R. G. R. BACON and D. J. MUNRO, J. chem. Soc. *1960*, 1339 yellow needles of m.p. 71–72 °C.

[14] This is considered the safest procedure, though dissolving *m*-XHQ and vitamin C simultaneously in water gave about the same results, judging from colour changes. Adding the vitamin C half an hour after dissolution of *m*-XHQ could not cancel a yellow colour change.

males were fed for 3 days with the test solutions and were then mated to the same number of untreated virgin females of the same age kept in cages with untreated milk and sugar. Eggs were collected daily and measured volumetrically, and hatching of eggs was checked on at least 6 occasions up to the age of 14 days of the females; the experiments were conducted at 27 °C. Some representative results, which could be confirmed in several replications, are given in the Table. The results indicate that while *m*-XHQ dissolved in water was inactive as a chemosterilant, *m*-XHQ with vitamin C in aqueous solution was as active as when dissolved in milk. Since, as mentioned above, the compound deteriorates also on dry storage, some further experiments were undertaken with strongly oxidized samples of already lilac colour (m.p. 127–128° instead of 150 °C of the pure substance, which is white). Such decomposed samples were nearly inactive when tested dissolved in milk at 0.15%, but when dissolved at the same concentration in water containing 0.3% vitamin C with formation of a colourless solution, they were reduced and reasonably if not completely reactivated (average corrected egg fertility, 12.5%).

We are unable to say whether this stabilization[15] would improve the antifertility effect of *m*-XHQ in mammals, which appears to be 'undramatic and somewhat difficult to demonstrate'[16], but perhaps endocrinologists might find it worthwhile to retest *m*-XHQ mixed with several times its weight of vitamin C, in rats[17,18].

Zusammenfassung. Nachweis, dass sich *m*-Xylohydrochinon (*m*-XHQ; 2,6-Dimethylhydrochinon) in wässriger Lösung zersetzt und so seine Wirkung als Insekten-Chemosterilant verliert. Auflösung der Substanz in Vitamin C enthaltenden wässrigen Lösungen verhindert den Vorgang.

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[15] According to S. N. SANYAL and S. S. GUHA SIRCAR, Med. Int. *27*, 53 (1963) the oral contraceptive *m*-XHQ tablets dispensed by the Indian Government Health Centres contain 'some citric acid used as antioxidant preservative'. We found that when *m*-XHQ was dissolved at a concentration of 0.15% in aqueous solutions of 0.075%, 0.15%, 0.2% or 0.3% citric acid, the solutions turned yellow nearly immediately and developed a strong smell. Within 1 day the yellow colour had become very intense. It was concluded that citric acid is unable to prevent the oxidation of *m*-XHQ in aqueous solutions. Little protection was afforded to *m*-XHQ in water by various mono- and disaccharides. Even a saturated (13:7) sucrose solution in water could not prevent the rapid oxidation of *m*-XHQ dissolved in it, but the protective effect of vitamin C was prolonged when a saturated sucrose solution was used as the vehicle.

[16] H. JACKSON, Pharmac. Rev. *11*, 135 (1959).

[17] We wish to thank Dr. S. N. SANYAL, Calcutta, for numerous samples of *m*-xylohydroquinone.

[18] Contribution from The National and University Institute of Agriculture, Rehovot, Israel. 1967 Series, No. 1126-E.

Nachweis von *n*-Butylamin in Äpfeln

Über das Vorkommen von wasserdampfflüchtigen primären Aminen in Apfelfrüchten berichteten HILKENBÄUMER et al.[1]. Bei den Apfelsorten «Cox's Orange», «Ontario» und «Jonathan» konnten Äthyl-, Isoamyl- und Hexylamin nachgewiesen werden.

Bei eigenen Versuchen über die Biogenese von flüchtigen Aminen konnten wir das Vorkommen von Äthyl- und Hexylamin bestätigen[2]. Isoamylamin dagegen liess sich nicht mit Sicherheit identifizieren. Nur in Einzelfällen, bei den Apfelsorten «Cox's Orange» und «Golden Delicious», konnte neben sehr geringen Mengen von vermutlich Propylamin und einem höheren Homologen des Hexylamins chromatographisch eine ninhydrinpositive Substanz in Spuren nachgewiesen werden, deren Rf-Wert mit dem von Isoamylamin übereinstimmte. Eine sichere Identifizierung war wegen der geringen Mengen nicht möglich. Ausserdem fanden wir ein zunächst unbekanntes Amin, das bei der papierchromatographischen Aufarbeitung (Trennung an Na-Acetat – vorbehandeltem Papier; Fliessmittel: *n*-Butanol-Wasser-Eisessig 50:49:1 (A) bzw. 50:40:10 (B))[3] einen geringfügig aber konstant höheren Rf-Wert als Isobutylamin aufwies und in allen Eigenschaften mit *n*-Butylamin übereinstimmte. Zur sicheren Identifizierung wurde das Amin im Vergleich zu den Butylaminen mikrokristallographisch nach der von KLEIN und STEINER entwickelten Methode mit 2,4-Dinitro-α-naphthol (DNN) untersucht[3–6]: Nach chromatographischer Trennung im Fliessmittelsystem A wurde die interessierende Zone, die ca. 10–20 µg Amin enthielt, aus

Chromatographische und mikrokristallographische Daten der Butylamine im Vergleich zu dem Amin aus Äpfeln

Amin	Rf-Werte		DNN-Verbindungen	
	A	B	Schmelzbereich[a]	Kristallform
Isobutylamin	0,60	0,72	155°–159 °C	Rhomben, prismatische Platten, Tafeln
n-Butylamin	0,66	0,76	147°–153 °C	Oft gekrümmte, lange Nadeln
«Apfelamin»	0,65	0,76	147°–152 °C	Wie *n*-Butylamin

[a] Die Schmelzpunkte variieren auch unter gleichen Bedingungen bei mehreren Einzelbestimmungen über einige Grade. Die aufgeführten Schmelzbereiche ergaben sich auf Grund von je 8 Einzelbestimmungen. Verwendete Apparatur zur Schmelzpunktbestimmung: Mikroskopheiztisch 350 (Leitz).

[1] F. HILKENBÄUMER, G. BUCHLOH und A. ZACHARIAE, Angew. Bot. *34*, 105 (1960).

[2] T. HARTMANN, Z. PflPhysiol., im Druck.

[3] E. STEIN v. KAMIENSKI, Diss. Bonn (1955).

[4] E. STEIN v. KAMIENSKI, Planta *50*, 291 (1957).

[5] G. KLEIN und M. STEINER, Jb. wiss. Bot. *68*, 602 (1928).

[6] B. ZORN und W. DIEHLMANN, Dte GesundhWes. *8*, 1522 (1953).