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Isolation of methylglyoxal from liver

(carbonyls/dicarbonyls/cellular regulation/acetaldehyde/2,4-dinitrophenylhydrazones)

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ABSTRACT **Acetaldehyde and methylglyoxal were shown to be present in liver bound to protein. They were isolated in the form of 2,4-dinitrophenylhydrazones and osazones, respectively. The NMR spectrum of pure methylglyoxal was recorded.**

Studies in our laboratories indicated that a dicarbonyl compound plays a major role in cellular regulations, but this dicarbonyl derivative was not identified. Our first attempt at isolation and identification (1) led to a ketoaldehyde, 3-deoxyglucosulose, which seemed to be an artifact (2). Later work focused attention on methylglyoxal. Kato and his associates (3) separated 23 carbonyl compounds from calf liver, which made it probable that the active ketoaldehyde could not be isolated without specific procedures. We took advantage of the fact that the methylglyoxal, if present in liver, would be bound to the structural proteins and so could be separated from the host of carbonyls by using, as starting material, liver homogenates from which the soluble carbonyl compounds had been eluted.

MATERIALS AND METHODS

A fresh, flash-frozen beef liver (5 kg) was blenderized with 4 liters of water. The pulp was adjusted to pH 4.5 and blenderized once more. The suspension was centrifuged and the supernatant, which contains hemolyzed blood and many soluble proteins, was discarded. The sediment was suspended in 5 liters of 1 M HCl and, under strong stirring 25 g of 2,4-dinitrophenylhydrazine was added. The suspension was vigorously stirred for 3 days. The fluid became yellow and, later, brown. In 2–3 days the color changed to reddish-brown. The pulp was then blenderized with 4 liters of ethyl acetate and centrifuged. The ethylacetate layer was washed with water, dried (Na_2SO_4), and concentrated under reduced pressure to a brown syrupy residue (140 g). This residue was first washed three times with 1 liter of petroleum ether (60–80°) and then three times with 500 ml of benzene. Evaporation of the solvents gave 118 g of petroleum ether extract and 20 g of benzene extract, respectively. The petroleum ether extract contained mostly canary yellow, highly lipophilic nitrophenylhydrazones.

UV spectra were taken with a Cary 14 spectrophotometer. For infrared spectra, Perkin-Elmer 137 and Beckman IR-8 spectrophotometers were used. NMR spectra were taken with EM-360 and T-60 MHz instruments.

RESULTS

Column chromatography of the benzene extracts over Silicagel, with benzene as eluent, yielded a compound which, recrystallized from benzene, had a melting point of 143° and was identified as 2,4-dinitrophenylhydrazone of acetaldehyde (4) by melting point; mixed melting point, and infrared and 60 MHz NMR spectra. UV: λ_{max} 252, 264, 359 nm. Infrared: 720, 940, 1080, 1160, 1220, 1310, 1330, 1378, 1460 (vs), 1590, 1620, 2900 (vs), 3300 cm^{-1}. NMR (acetone-d_6): δ 2.01 (^3H, d, H_3C-CH); δ 7.7–8.0 [^3H, m, aryl H-6, NH and C-H (aldehyde)]; δ 8.25 (^1H, *s*, aryl H-5); δ 8.85 (^1H, d, J 1.5 Hz, H-3 aryl). The doublet at δ 2.01 collapses to a singlet upon irradiation at δ 8.0.

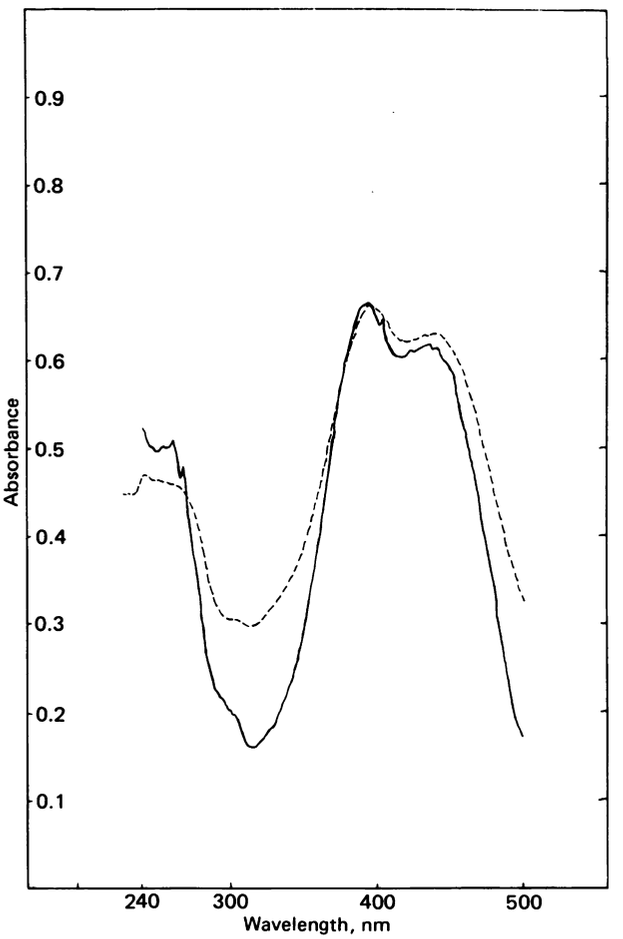

FIG. 1. UV spectra of methylglyoxal bis-2,4-dinitrophenylhydrazones (osazones) from liver (solid curve) and the synthetic product (in chloroform) (broken curve).

Further elution with acetone/benzene (0.1:9.9, vol/vol) yielded a reddish-brown residue (4 g) which, upon repeated washing with acetone, gave an insoluble residue (0.8 g). Preparative thin-layer chromatography on Silicagel with benzene/pyridine (9.9:0.1, vol/vol) gave a compound, crystallized from dimethylformamide as orange crystals, melting point 306°. The R_F values of these crystals in benzene/pyridine (9.9:0.1) and chloroform/benzene (1:2) were identical with

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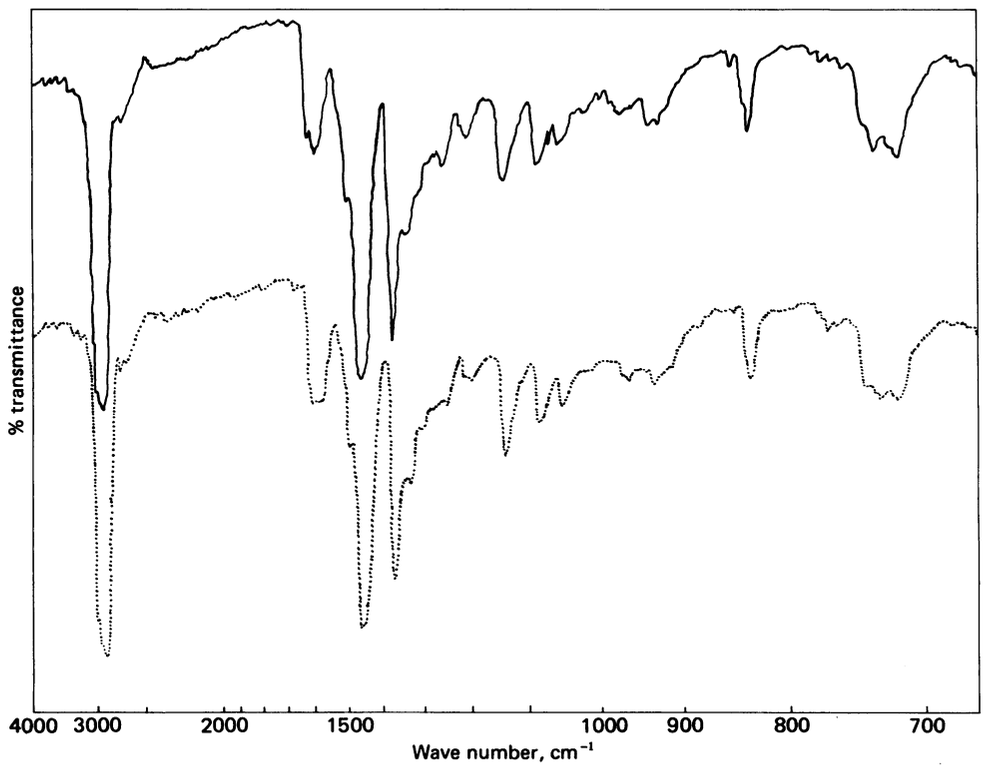

FIG. 2. Infrared spectra of the 2,4-dinitrophenylosazones of natural (solid curve) and of synthetic (broken curve) methylglyoxal (in Nujol mull).

those of authentic bis-2,4-dinitrophenylhydrazone of methylglyoxal. Neuberg and Kobel (5) reported a melting point of 309° for "synthetic" methylglyoxal dinitrophenylhydrazone.

Both the UV (Fig. 1) and infrared spectra (Fig. 2) of the natural and synthetic products were superimposable. The NMR spectrum of the osazone could not be taken because of poor solubility in the usual solvents. The elemental analysis of the sample obtained from liver tissue agreed with the calculated values. (Analysis: Found C, 41,90; H, 2.90; N, 25.80. $C_{15}H_{12}O_8N_8$ requires C, 41.60; H, 2.78; N, 25.90.) Thus, the experiment showed that methylglyoxal is a natural constituent of liver tissue.

Finally, we present the NMR spectrum of synthetic methylglyoxal (Fig. 3) in anhydrous, monomeric form, taken in carbon tetrachloride. The aldehyde methine proton resonated at δ 9.0(s) and the methyl protons at 2.2(s); the integration showed the expected perfect 1:3 ratio. However, if the same sample of methylglyoxal was dissolved in ^2H_2O, a very complex spectrum resulted (Fig. 4). The aldehydic proton moved into the δ 5–6 region as a consequence of hydration of the aldehyde group. However, the complex pattern in the methyl region and the appearance of a strong peak at δ 2.70 indicate involvement of the ketonic carbonyl group in hydration; the presence of dimeric hydrate structures cannot be excluded either.

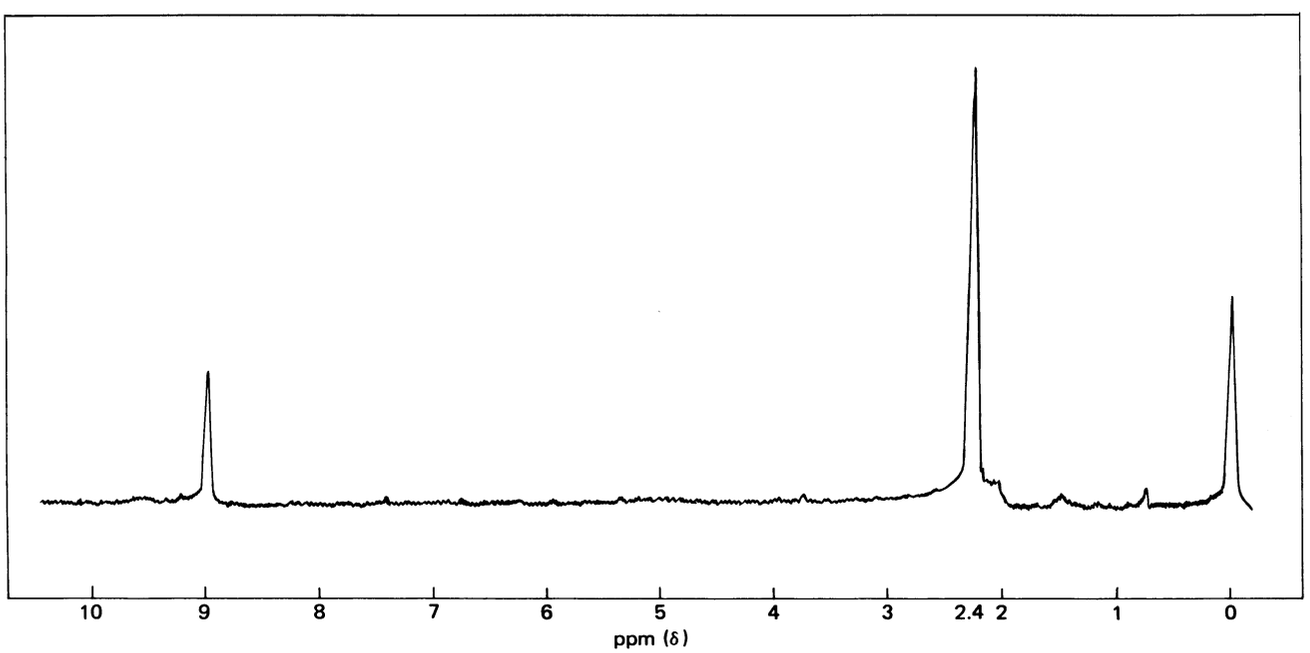

FIG. 3. NMR spectra (60 MHz) of anhydrous, monomeric methylglyoxal (in carbon tetrachloride).

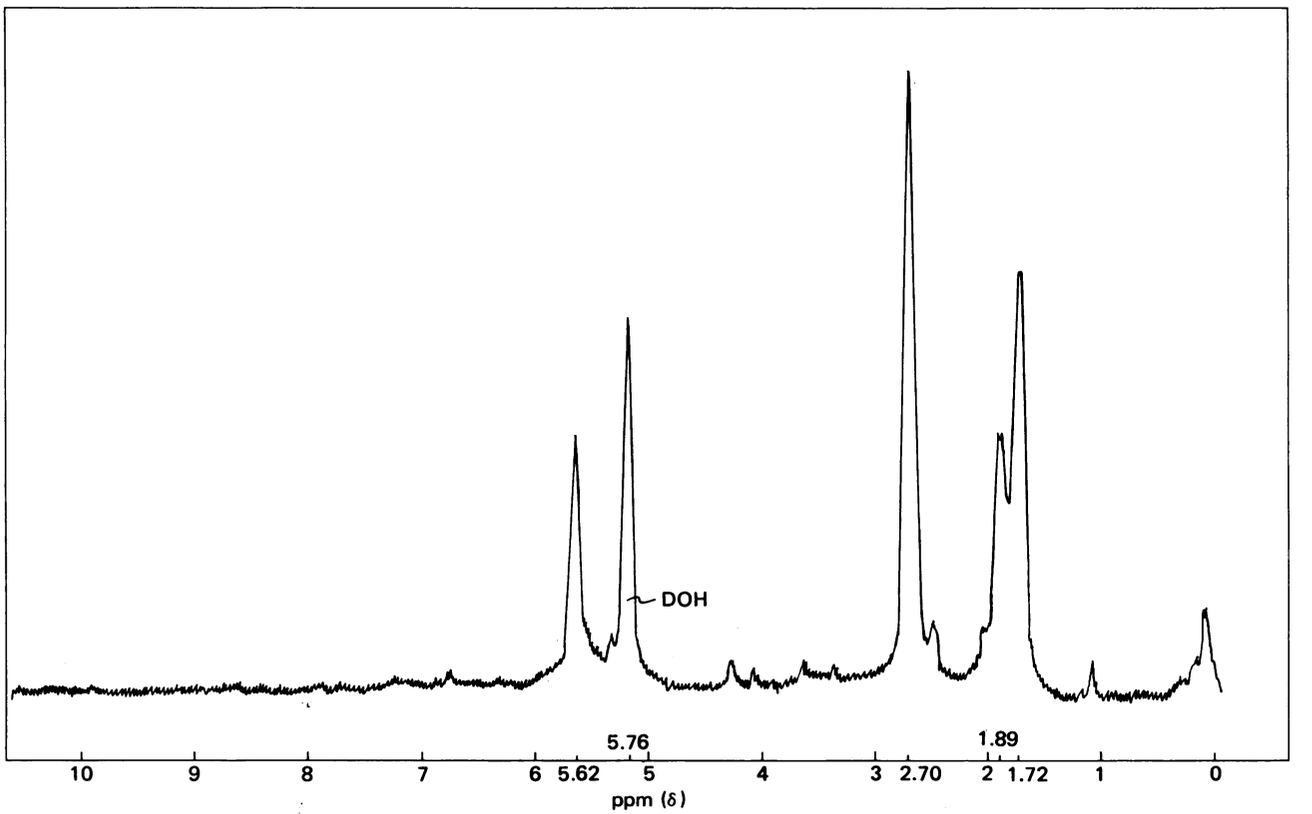

FIG. 4. NMR spectrum (60 MHz) of synthetic monomeric methylglyoxal dissolved in ^2H_2O.

1. Fodor, G., Sachetto, J. P., Szent-Györgyi, A. & Együd, L. G. (1967) *Proc. Natl. Acad. Sci. USA* **57**, 1644–1650.
2. Otsuka, H. & Együd, L. G. (1968) *Biochim. Biophys. Acta* **165**, 172–173.
3. Kato, H., Tsusaka, N. & Fujimaki, M. (1970) *Agric. Biol. Chem. (Japan)*, **34**, 1541–1548.
4. Purgotti, A. (1894) *Gazz. Chim. Ital.* **24**(I), 554–584.
5. Neuberg, C. & Kobel, M. (1928) *Biochem. Z.* **203**, 463–468.