

Girard *T* Reagent for Carbonyls

THE water-soluble properties of Girard *T* hydrazones make the reagent potentially effective for isolation of carbonyls from mixtures soluble in non-polar solvents. In work on the chemistry of rancidity¹, we have found the reagent useful in learning about the distribution of volatile and non-volatile carbonyls in rancid fat. This work indicated possible usefulness of the reagent for investigation of the complex carbonyl anatomy of autoxidized fats. Successful application depends on adaptation to quantitative isolation of very small amounts of carbonyls, and on devising means of controlling the reaction so as to isolate at will free carbonyl and bound or potential carbonyl.

This report deals with certain aspects of the methodology of the Girard reaction. In experiments with known amounts of monocarbonyls, 0.5-1.0 gm. of Girard *T* reagent was reacted in 20.0 ml. of purified anhydrous methanol or ethanol and shaken 3 hr. at room temperature, or refluxed for 1 hr. The reaction mixture was poured into 200 ml. of ice water, and then extracted with petroleum ether to remove non-carbonylic material. The aqueous phase contained the Girard *T* hydrazones. These were converted directly into 2,4-dinitrophenylhydrazones by treatment with 30 ml. of a saturated 2,4-dinitrophenylhydrazine

Table I. BLANK DETERMINATIONS

Girard <i>T</i> reagent	2,4-Dinitrophenylhydrazine	Ethanol	Tertiary butyl alcohol	No solvent
—	RT* 1 hr.	0.048	—	—
—	RT 24 hr.	0.130	0.024	—
—	Heated 1 hr.	0.480	—	—
0.5 gm. (unpurified) RT, 3 hr.	RT 24 hr.	—	0.250	—
1.0 gm. (unpurified) RT, 3 hr.	RT 24 hr.	0.850	0.518	—
0.5 gm. (purified) RT, 3 hr.	RT 24 hr.	0.338	0.050	—
0.5 gm. (purified) Reflux, 1 hr.	RT 24 hr.	0.478	0.120	—
0.5 gm. (purified) Reflux, 2 hr.	RT 24 hr.	0.900	—	—
0.5 gm. (purified) RT, 3 hr.	Heated 1 hr.	2.63	0.813	0.087
0.5 gm. (purified) RT, 3 hr.	Heated 1 hr.	—	0.310 †	—

* Room temperature.

† Purified by treatment with 2,4-dinitrophenylhydrazine and hydrochloric acid.

solution in 2 N hydrochloric acid² and 190 ml. of water. After standing overnight at room temperature, or heating 1 hr. on the steam bath, the 2,4-dinitrophenylhydrazones were removed by successive extractions with carbon tetrachloride and benzene. Monocarbonyl hydrazones were separated by passage of extract residues through hydrated alumina². Total amounts of these compounds were determined by measurement of absorbance at the wave-length of maximum absorption³⁻⁶ in 100 ml. of carbon tetrachloride solution. In the case of mixtures, paper chromatographic methods are available for resolution, identification, and estimation of monocarbonyls³⁻⁶.

The above procedure for the Girard reaction gave high blanks which seriously affected determinations of very small amounts of monocarbonyls. The Girard *T* reagent was found to contain hydrazone, which could be removed by several recrystallizations under mild conditions from ethanol. However, the greater part of the blank was found to result from a curious reaction between the primary alcohols used as solvents and both the Girard *T* and 2,4-dinitrophenylhydrazine. The amount of formaldehyde or acetaldehyde hydrazone formed was related directly to the severity of reaction conditions and independent of exposure to air. Apparently, this carbonyl formation is a dehydrogenation and analogous to the formation of osazones from α -keto alcohols. The reaction has been studied on a very large scale by Braude and Forbes⁷, who determined that it resulted in a 5-25 per cent yield with primary and secondary alcohols containing two or more ethylenic or phenyl substituents in conjugation with the alcohol group. These investigators stated that derivatives were not formed with monophenyl, α,β unsaturated and saturated alcohols. This has been found not to be completely correct in the case of the two saturated primary alcohols used.

Methanol and ethanol have been the standard solvents used in the Girard *T* reaction since it was first reported⁸. Tertiary butyl alcohol is proposed as a relatively inert solvent for use in the presence of the Girard *T* reagent and 2,4-dinitrophenylhydrazine. The Girard *T* reagent has limited, but adequate, solubility in this alcohol, which is a much better solvent for fats than methanol or ethanol. Blanks found with ethanol and tertiary butyl alcohol are shown in Table 1. Ethanol blanks were substantial, and increased with vigour of conditions of reaction. Both Girard *T* and 2,4-dinitrophenylhydrazine are shown to act on ethanol. Purified butyl alcohol gave negligible blanks when the Girard *T* reagent was recrystallized, except when heated in the 2,4-dinitro-

phenylhydrazine step. Purification of tertiary butyl alcohol with 2,4-dinitrophenylhydrazine and hydrochloric acid reduced this blank somewhat. This may indicate the presence of a persistent impurity, possibly a ketal. In any event, heating in the 2,4-dinitrophenylhydrazine step was unnecessary for complete conversion.

The use of tertiary butyl alcohol in the Girard *T* reaction gave nearly quantitative results, under mildest conditions of reaction, with *n*-alkanals, *n*-alk-2-enals, and *n*-alk-2,4-dienals. Reaction of methyl ketones with the Girard *T* reagent was less complete (about 80 per cent).

The entire suitability of the Girard *T* reagent for study of total carbonyls in autoxidized fat remains to be determined. Little is known about its reaction with dicarbonyl compounds. In view of its dehydrogenation action on primary and secondary alcohols, the reagent might convert hydroxy compounds produced in the autoxidation into carbonyls. Direct reaction of Girard *T* reagent or 2,4-dinitrophenylhydrazine with fat autoxidation products could result in higher total carbonyl than actually present. There has been some indication that this may occur; such as continual removal of carbonyl on repeated treatments with Girard *T* reagent¹, but further study is necessary. It was considered that the information in this report might be of use to those interested in applications of the Girard *T* reagent.

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