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# Short Communications

## Preparation of *tert*-Butylmaleic Acid and its Di-*tert*-butyl Ester

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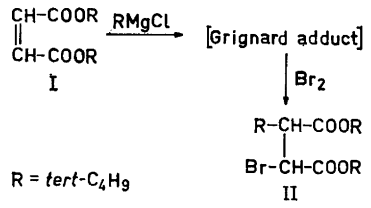
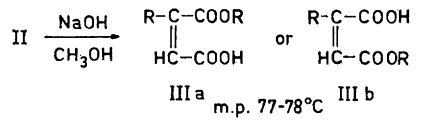
In connection with our work on the reaction between Grignard reagent and alkyl substituted maleic esters,[1-4] we have synthesized the previously unknown *tert*-butylmaleic acid.

The usual synthetic way to alkyl substituted maleic acid derivatives from the corresponding α-alkyl acetoacetic esters [5] is not useful here, as α-*tert*-butylacetoacetic ester is not readily accessible. However, it is known that adducts from Grignard reagents and α,β-unsaturated esters react with halogens to form α-halo esters.[6-8] This reaction is utilized here, letting the adduct from *tert*-butylmagnesium chloride and di-*tert*-butylmaleinate react with bromine according to *scheme 1*.




Scheme 1

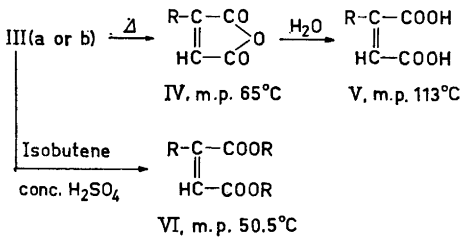
The alkyl-bromosuccinate (II) consists of a mixture of the diastereomers (see *experimental*), but treatment with methanolic sodium hydroxide yields exclusively one of the mono-*tert*-butyl *tert*-butylmaleinates (IIIa *or* IIIb) in an almost quantitative yield (scheme 2).




Scheme 2

It is difficult to explain the facts, 1) that only the maleic acid derivative is formed, and 2) that one of the ester groups is quantitatively cleaved. A reaction mechanism explaining both these facts would be the most satisfying, but knowledge of which of the ester groups hydrolyzes is a precondition. NMR gives no information on this point, as the vinylic proton of the mono-ester (III) absorbs at 4.18 ppm, while the vinylic protons of the acid (V) and the diester (VI) absorbs at 4.08 and 4.28 ppm, respectively.

The mono-ester is converted to the acid *via* the anhydride and to the di-ester in good yields (scheme 3).




Scheme 3

The overall yield of *tert*-butylmaleic acid from di-*tert*-butylmaleinate is 37 %, but can be increased to 62 % if the bromo-ester (II) is not purified (see *experimental*). The

overall yield of di-*tert*-butyl *tert*-butylmaleinate is 29 %.

*Experimental. Di*-tert-*butylmaleinate* (I) was synthesized in a 60 % yield from maleic acid and isobutene as described for di-*tert*-butyl malonate.[9] The compound was recrystallized from ether, m.p. 68°C. (Found: C 63.10; H 8.86. Calc. for $C_{12}H_{20}O_4$: C 63.13; H 8.83). NMR-spectrum: 3.91 ppm (*s* CH=C) and 8.50 ppm (*s* $OC(CH_3)_3$).

*Di*-tert-*butyl* α-tert-*butyl*-α'-*bromosuccinate* (II). *tert*-Butyl-magnesium chloride was prepared according to *Organic Syntheses*,[10] and left standing to the next day. The coloured impurities were thus precipitated, and the colourless solution decanted to a stock container and titrated (halogen and base).

Without special precautions 0.25 mole of the Grignard reagent was transferred to a three-necked flask with thermometer, dropping funnel and drying tube. Dry ether was added to a total volume of 250 ml, and the flask cooled to −10°C in a $CO_2$-acetone bath. 45.6 g I dissolved in 200 ml ether was added as fast as possible (*ca.* 5 min), keeping the temperature below 0°C. When the ester had been added, the cooling bath was removed and the reaction mixture left at *ca.* 0°C for 15 min. Then it was cooled to −10°C again, and 40 g dry bromine was added as fast as possible (*ca.* 3 min) again keeping the temperature below 0°C. A saturated sodium sulfite solution was added until the bromine colour disappeared, and the reaction mixture then poured into excess cold 2 N hydrochloric acid. The phases were separated, and the organic phase washed with alkali twice (all traces of acid must be removed before distillation) and then water. After drying, the ether was removed and the residue fractionated to give a forerun of 10.5 g (18 %) di-*tert*-butyl *tert*-butylsuccinate[4] b.p. 85−90°C/1 mm, followed by II, which was collected at 105−115°C/1 mm. The yield is 30.5 g (42 %), the main part boiling at 109°C/1 mm, $n_D^{20}$ = 1.4558. (Found: C 52.75; H 7.83; Br 22.00. Calc. for $C_{16}H_{29}BrO_4$: C 52.60; H 8.00; Br 21.87).

The product is a mixture of diastereomers (*ca.* 2:1 based on the NMR-absorptions of the α-*tert*-butyl groups). NMR-spectrum: For one of the diastereomers 5.63 ppm (*d* α-CHBr) and 7.10 ppm (*d* α-CH) ($J$ = 10 cps) and for the other 5.57 ppm (*d* α-CHBr) and 6.98 ppm (*d* α-CH) ($J$ = 12 cps). 8.45−8.55 ppm (two pairs of $OC(CH_3)_3$). 8.95 and 8.84 ppm (two singlets, two different α-$C(CH_3)_3$).

*Mono*-tert-*butyl* tert-*butylmaleinate* (IIIa or b). 13.5 g II was refluxed for 2 h with 3 equiv. of sodium hydroxide in 100 ml methanol. The methanol was then distilled and the residue dissolved in water. The solution was extracted with ether, acidified, and extracted with ether again; after drying and removing the ether, III was obtained as an oil which solidified overnight; 9.5 g (99 %). Recrystallization from petroleum ether (40/60) yielded the pure acid as colourless crystals, m.p. 77−78°C. (Found: C 62.90; H 8.85. Calc. for $C_{12}H_{20}O_4$: C 63.13; H 8.83). NMR-spectrum: −2.09 (*s* COOH), 4.18 (*s* $OC(CH_2)_3$) and 8.79 ppm (*s* $C(CH_3)_3$).

tert-*Butylmaleic anhydride* (IV). 9.5 g III was heated cautiously in a rotating flask with a Bunsen flame until the evolution of isobutene and water stopped. By cooling, the residue crystallized readily. Crystallization from petroleum ether yielded 5.6 g IV (88 %), in slightly brownish flakes, m.p. 60−62°C. Recrystallization gave the compound in colourless flakes, m.p. 65°C. (Found: C 62.03; H 6.42. Calc. for $C_8H_{10}O_3$: C 62.32; H 6.54). NMR-spectrum: 3.40 ppm (*s* CH=C) and 8.62 ppm (*s* $C(CH_3)_3$).

tert-*Butylmaleic acid* (V). 5.6 g IV was dissolved in 30 ml water with 4.5 g potassium hydroxide. After acidifying, the organic compound was taken up in ether, dried, and the ether evaporated *in vacuo*. Yield 6.4 g (quant.). Recrystallization from chloroform/petroleum ether (1:4) gave the pure acid, m.p. 113°C. (Found: C 55.65; H 7.29. Calc. for $C_8H_{12}O_4$: C 55.80; H 7.03). NMR-spectrum: −0.84 (*s* COOH), 4.08 (*s* CH=C) and 8.76 ppm (*s* $C(CH_3)_3$).

A better overall yield can be obtained if the crude bromo-ester (II), after evaporation of the ether, is treated with base. Both the resulting mono-ester (III) and the anhydride (IV) is then contaminated with some of the corresponding saturated compound. But a simple distillation of IV, followed by a crystallization from petroleum ether gives the pure anhydride (m.p. 64°C) in a 62 % yield from I.

*Di*-tert-*butyl* tert-*butylmaleinate* (VI) was prepared from 21.9 g III similar to I.[9] Yield 19.0 g (70 %) semisolid ester (VI). Recrystallization from petroleum ether gave the pure VI, m.p. 50.5°C, in 78 % yield. (Found: C 67.90; H 9.98. Calc. for $C_{16}H_{28}O_4$: C 67.57; H 9.93). NMR-spectrum: Singlets 4.28 (CH=C), 8.47, 8.53 (two $OC(CH_3)_3$) and 8.82 ppm ($C(CH_3)_3$).

All melting and boiling points are uncorrected.

The NMR-spectra were recorded on a Varian A-60 instrument; $CDCl_3$ used as solvent with the values given in τ-units with TMS as internal standard.

Microanalysis by Mr. Preben Hansen, Chemical Laboratory II, University of Copenhagen.

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# Intestinal Dipeptidases

## IX. Studies on Dipeptidases of Human Intestinal Mucosa

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Recently a spectrophotometric method for assaying dipeptidase activity has been described.[1] The method has been employed for investigation of various intestinal dipeptidases, including the activities against L-alanyl-L-glutamic acid, L-alanyl-L-proline, glycylglycine, glycyl-L-leucine, and glycyl-L-valine, in different species (for ref. see 2).

Because of interest of covering the dipeptidases performing the hydrolysis of dipeptides containing glutamic acid and proline, issued from studies of gastrointestinal disorders, additional reactions have now been included in the investigation. The present report describes the results obtained from studies of the L-glutamyl-L-proline, L-glutamyl-L-valine, L-valyl-L-glutamic acid, and L-valyl-L-proline dipeptidase activities in the human small intestine.

The dipeptides, L-glutamyl-L-proline, L-glutamyl-L-valine, L-valyl-L-glutamic acid, and L-valyl-L-proline, were all products of YEDA, Rehovoth, Israel. For the assays they were used in aqueous solutions in the following concentrations: 0.008 M L-glutamyl-L-proline; 0.03 M L-glutamyl-L-valine; 0.03 M L-valyl-L-glutamic acid; 0.006 M L-valyl-L-proline. The corresponding amino acids (Mann Research Labs., New York) were used in aqueous solutions in combinations and concentrations as follows: 0.008 M L-glutamic acid and 0.008 M L-proline; 0.03 M L-glutamic acid and 0.03 M L-valine; 0.006 M L-valine and 0.006 M L-proline. The assay procedure was the same as described previously.[1] As enzyme solution served human mucosal homogenates prepared as described elsewhere.[3] The mucosa was scraped off from operation specimens, taken from the distal part of the ileum of four adult humans.[3]

The effect of pH on the different dipeptidase reactions was studied over a pH range from 5.0 to 9.5 by using 0.15 M phosphate and borate buffer solutions. The pH-optima of the four reactions differed slightly from each other (Fig. 1). They were found to be 7.2 for L-glutamyl-L-proline dipeptidase, 6.8 for L-glutamyl-L-valine dipeptidase, 7.9 for L-valyl-L-glutamic acid dipeptidase, and 6.7 for L-valyl-L-proline dipeptidase.

*Table 1.* Effect of bivalent metal ions (final concentration $5.9 \times 10^{-5}$ M) on dipeptidase activities of the small intestine of adult human. Optimum pH.

| Substrate | Relative rate of hydrolysis [a] in presence of metal ions | | | |
|---|---|---|---|---|
| | $Co^{2+}$ | $Mn^{2+}$ | $Mg^{2+}$ | $Zn^{2+}$ |
| L-Glutamyl-L-proline | 0.9 | 0.9 | 1.0 | 1.0 |
| L-Glutamyl-L-valine | 0.3 | 0.5 | 0.6 | 0.1 |
| L-Valyl-L-glutamic acid | 0.7 | 1.0 | 0.9 | 0.7 |
| L-Valyl-L-proline | 1.2 | 1.2 | 1.2 | 1.3 |

[a] The rate of hydrolysis without metal ions added taken as 1.