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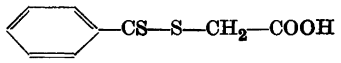
On Carboxymethyl Dithiophenylacetate and Its Reactions with Amines, Amino Acids and Peptides

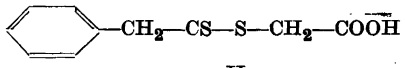
ANDERS KJÆR

Chemical Laboratory, University of Copenhagen, Copenhagen, Denmark

In a previous communication [1] the applicability of carboxymethyl dithiobenzoate (I) as a means of introducing the thiobenzoyl-grouping in esters and amides of α-amino acids was demonstrated. The present paper describes the preparation of the heretofore unknown, analogous carboxymethyl dithiophenylacetate (II), which has proved to be a very convenient reagent for introducing the phenylthioacetyl-grouping in amines, amino acids and peptides.



I

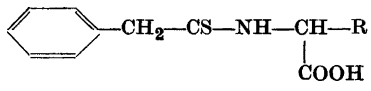


II

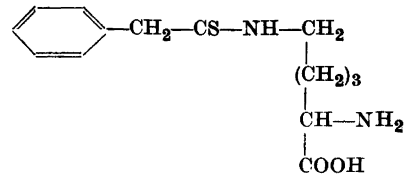
Phenylthioacetylation of amino acids has formerly been achieved by means of sodium dithiophenylacetate [2], although in poor yields (20–25 %). Better results were obtained when methyl dithiophenylacetate was employed [3], a procedure recently adopted by other authors [4,5].

Upon reaction of benzylmagnesium chloride with carbon disulphide and chloroacetic acid, carboxymethyl dithiophenylacetate (II) was produced as an intensely yellow compound, separating from hexane in a total yield of 60–70 %. The acid could be stored for months in a closed vessel with no signs of deterioration. Its reactions with ammonia, morpholine and aniline proceeded with great ease at room temperature, rendering the substituted thioamides in satisfactory yields. Similarly, reaction of the dithioacid with glycine, L- and DL-alanine, L-valine, DL-leucine, L-isoleucine and DL-methionine afforded the respective phenylthioacetyl-derivatives (III) as colourless crystalline compounds, while the corresponding oily reaction products of DL-phenylalanine, L-leucine and DL-valine were not obtained in crystalline form. No reaction was noticed with α-aminoisobutyric acid, the failure being

ascribable to sterically unfavorable conditions as suggested previously in the case of thiobenzoylation. When DL-lysine was treated with one mole of the dithioacid (II), a mono-thioacylated derivative was obtained for which the structure (IV) is tentatively suggested, on the basis of the finding that the substance produced a positive ninhydrin reaction. On repeating the experiment with three moles of dithioacid, an oil was obtained which yielded the *mono*-derivative (IV) as the only crystalline constituent.



III



IV

In order to extend the reaction to amino amides and peptides, glycinamide and L-tyrosinamide were successfully submitted to phenylthioacetylation. Again, well crystallizing derivatives were obtained from glycylglycine, glycylglycylglycine and DL-alanylglycine. Glycyl-L-tryptophane yielded a crystalline product, which could not be obtained in analytically pure form.

L-Asparagine gave an 80 % yield of the phenylthioacetyl-derivative, which was selected to prove the sterical homogeneity of the thioacylated compounds. Oxidative desulphurization with hydrogen peroxide in slightly alkaline solution provided N-phenylacetyl-L-asparagine, whose dextrorotation practically agreed with that of a synthetic specimen, prepared by phenacetylation of L-asparagine under non-racemizing conditions.

From the experimental evidence presented above, it may be concluded that carboxymethyl dithiophenylacetate can generally be used as a reagent for introducing the phenylthioacetyl-grouping in amines, amino acids and peptides. The reactions proceed without racemization at room temperature and in satisfactory yields.

EXPERIMENTAL *

Carboxymethyl dithiophenylacetate (II)

A Grignard-solution, prepared under nitrogen from 16.5 g of magnesium (0.68 mole), 82.2 g of benzyl chloride (0.65 mole) and 300 ml of dry ether, was added during one hour to a well agitated and ice-cooled solution of 76.5 g of dry carbon disulphide (1.01 mole)

* All melting points are uncorrected and determined in capillary tubes in an electrically heated bloc.

in 150 ml of dry ether. The mixture was kept overnight, thus acquiring room temperature. Then the solution was poured onto 400 g of crushed ice and the aqueous layer separated from the ethereal phase which contained a voluminous amorphous precipitate. A solution of 64.2 g of chloroacetic acid (0.68 mole) and 38 g of sodium carbonate in 250 ml of water was added in one portion to the aqueous phase, and within a few minutes, the mixture was transformed into a yellow mass of fine needles, consisting of the sodium salt of (II). After standing at 0° for 48 hours, a mixture of 45 ml of concentrated sulphuric acid (0.83 mole) and 50 ml of water was added dropwise to the crystal slurry in order to liberate the free dithioacid. A small amount of a brownish, oily substance separated and was taken up in ether. After thorough extraction with several portions of fresh ether, all ether extracts were pooled, dried over sodium sulphate and the ether removed, leaving the final product as a yellow crystalline mass. Recrystallized from ligroin, 87 g (64 %) of carboxymethyl dithiophenylacetate was obtained. M. p. 78—79°.

A sample for analysis was prepared by an additional crystallization from ligroin. M. p. 79—80°.

$C_{10}H_{10}O_2S_2$ (226.30)	Calc.	C 53.07	H 4.45	S 28.33
	Found	» 53.03	» 4.56	» 27.99

Neutralization equivalent found by microtitration (indicator : phenolphthalein) : 225.

Phenylthioacetylation of amines, α-amino acids, amino amides and peptides

Phenylthioacetamide. Separated as prisms from a solution of 595 mg of (II) in 10 ml of dilute aqueous ammonia. Yield 321 mg (81 %). M. p. 98°. (Lit.[6] 97.5—98°).

Phenylthioacetanilide. To a solution of 667 mg of (II) in 2.95 ml of 1 *N* NaOH was added 0.5 ml of aniline, whereupon an oil separated which rapidly became crystalline. Yield 641 mg (96 %). Recrystallized from benzene-hexane as pale yellow needles. M. p. 89°. (Lit.[7] 87°).

Phenylthioacetomorpholide. From a solution of 456 mg of (II) in 2.02 ml of 1 *N* NaOH containing 0.25 ml of morpholine, an oil separated which on cooling gave 434 mg (97 %) of crystalline material. Recrystallized from dilute methanol as compact, colourless rhombs. M. p. 79—80°. (Lit.[8] 79—80°).

N-Phenylthioacetylglycine (III, R = H). A mixture of 2.26 g of (II) and 0.75 g of glycine in 10.0 ml of 2 *N* NaOH after standing and acidification deposited 1.80 g (86 %) of pale yellow plates. Recrystallized from water, the colourless compound melted at 141—142°. (Lit.[2] 142°).

N-Phenylthioacetyl-L-alanine (III, R = CH_3). To 0.84 g of L-alanine, dissolved in 18.8 ml of 1 *N* NaOH was added 2.13 g of (II). Acidification gave an oil which only after prolonged cooling crystallized partly. Twice recrystallized from ether-petroleum ether, the substance melted at 121—122°.

$C_{11}H_{13}O_2NS$ (223.28)	Calc.	N 6.27	S 14.36
	Found	» 6.32	» 14.34

Neut.equiv. found 222 (microtitration).

Rotation: $[\alpha]_D^{23} = -2.07°$ (c = 2.5 g pr. 100 ml of solution; methanol).

N-Phenylthioacetyl-DL-alanine (III, R = CH_3). Prepared exactly as described for the L-isomeride. Yield 75 %. M. p. 121°. (Lit.[2] 121°).

N-Phenylthioacetyl-L-valine (III, R = *iso*-C_3H_7). From 0.53 g of L-valine and 1.02 g of (II) in the usual way. The oily, crude material crystallized on keeping, yielding 0.81 g

(72 %) of a yellowish product. Crystallized from ether-petroleum ether in irregular, colourless plates. M. p. 100—101°.

$C_{13}H_{17}O_2NS$ (251.34)	Calc.	N	5.70	S	12.76
	Found	»	5.67	»	12.79

Neut.equiv. found 250 (microtitration).

Rotation: $[\alpha]_D^{23} = -13.80°$ ($c = 1.5$; methanol).

The corresponding DL-isomeride could not be obtained in crystalline form during this investigation, but has been recorded previously [4] with m. p. 102—103°.

N-Phenylthioacetyl-DL-leucine (III, R = *iso*-C_4H_9). Prepared from 262 mg of DL-leucine and 452 mg of (II) in 4.00 ml of 1 *N* NaOH. After acidification, the oily product was extracted with ether, which after drying was removed by spontaneous evaporation, leaving 420 mg (78 %) of a crystalline product. This, recrystallized from ether-petroleum ether for analysis, had m. p. 111°.

$C_{14}H_{19}O_2NS$ (265.36)	Calc.	N	5.28	S	12.08
	Found	»	5.36	»	12.00

Neut.equiv. found 267 (microtitration).

N-Phenylthioacetyl-L-isoleucine (III, R = *sec.*-C_4H_9). From 456 mg of L-isoleucine and 790 mg of (II) in 7.00 ml of 1 *N* NaOH in the usual way. Yield 702 mg (76 %). Colourless, dense prisms from chloroform-petroleum ether. M. p. 95—96°.

$C_{14}H_{19}O_2NS$ (265.36)	Calc.	N	5.28	S	12.08
	Found	»	5.48	»	11.93

Neut.equiv. found 265 (microtitration).

Rotation: $[\alpha]_D^{23} = -7.70°$ ($c = 1.5$; methanol).

The corresponding DL-isomeride has previously been reported [4]; m. p. 95—96°.

N-Phenylthioacetyl-DL-methionine (III, R = $CH_3SCH_2CH_2$). Treated in the usual way, a mixture of 450 mg of DL-methionine and 682 mg of (II) in 6.05 ml of 1 *N* NaOH yielded on acidification 640 mg (75 %) of crystalline material. Colourless plates from chloroform-petroleum ether. M. p. 117—119°.

$C_{13}H_{17}O_2NS_2$ (283.40)	Calc.	N	4.94	S	22.63
	Found	»	5.03	»	22.55

Neut.equiv. found 282 (microtitration).

ε-(N-Phenylthioacetyl)-DL-lysine (IV). To a solution of 1.02 g of lysine monohydrochloride in 16.8 ml of 1 *N* NaOH was added 1.26 g of (II). On acidification, 0.88 g (56 %) of crystalline material separated after triturating the crude reaction mixture with hot ethanol. M. p. 250° (dec.). The compound produced an intensively violet colour on short heating with ninhydrin.

$C_{14}H_{20}O_2N_2S$ (280.38)	Calc.	N	9.99	S	11.44
	Found	»	9.86	»	11.37

N-Phenylthioacetyl-glycinamide. A smooth reaction ocurred upon mixing 0.90 g of glycinamide hydrogen sulphate [9] and 1.19 g of (II) in 15.7 ml of 1 *N* NaOH, immediately followed by the separation of 0.86 g (79 %) of practically colourless plates. Recrystallized twice from chloroform-petroleum ether for analysis. M. p. 104—105°.

$C_{10}H_{12}ON_2S$ (208.28)	Calc.	N	13.45	S	15.39
	Found	»	13.45	»	15.54

N-Phenylthioacetyl-L-tyrosinamide. To a solution of 442 mg of (II) in 2.00 ml of 1 *N* NaOH was added 351 mg of L-tyrosinamide dissolved in 3.5 ml of lukewarm water. After

a few minutes, 469 mg (77 %) of a crystalline powder could be collected. Separated as stout prisms from dilute methanol. M. p. 88—90°.

$C_{17}H_{18}O_2N_2S$ (314.39) Calc. N 8.91 S 10.20
Found » 8.86 » 10.14

Rotation: $[\alpha]_D^{23} = +77.69°$ ($c = 2.5$; methanol).

N-Phenylthioacetyl-glycylglycine. A mixture of 0.93 g of glycylglycine hydrochloride monohydrate and 1.13 g of (II) in 15.0 ml of 1 *N* NaOH was acidified, when 0.75 g (56 %) of crystals separated, which after recrystallization from chloroform-petroleum ether melted at 157—158° (dec.).

$C_{12}H_{14}O_3N_2S$ (266.31) Calc. N 10.52 S 12.04
Found » 10.48 » 11.93

Neut.equiv. found 268 (microtitration).

N-Phenylthioacetyl-glycylglycylglycine. Similarly, after dissolution of 0.84 g of triglycine and 1.00 g of (II) in 8.9 ml of 1 *N* NaOH, acidification yielded 0.93 g (65 %) of a crystalline product, which could be purified by recrystallization from chloroform-petroleum ether. M. p. 166—167° (dec.).

$C_{14}H_{17}O_4N_3S$ (323.36) Calc. N 12.99 S 9.92
Found » 12.96 » 9.71

Neut.equiv. found 321 (microtitration).

*N-Phenylthioacetyl-*DL*-alanylglycine.* From a solution of 420 mg of DL-alanylglycine and 650 mg of (II) in 5.74 ml of 1 *N* NaOH, addition of excess acid precipitated 570 mg (71 %) of the crude reaction product. Recrystallized once from chloroform-petroleum ether for analysis. M. p. 181—182° under evolution of gas.

$C_{13}H_{16}O_3N_2S$ (280.34) Calc. N 9.99 S 11.44
Found » 9.91 » 11.52

Neut.equiv. found 277 (microtitration).

*N-Phenylthioacetyl-*L*-asparagine* (III, R = CH_2CONH_2). To 1.62 g of L-asparagine monohydrate in 10.8 ml of 1 *N* NaOH, a solution of 2.44 g of (II) in 10.8 ml of 1 *N* NaOH was added. After standing for 20 hours, cooling and acidification yielded 2.42 g (84 %) of practically pure crystalline substance. Separated from ethyl acetate-petroleum ether in beautiful, colourless plates. Dried for 18 hours at room temperature over P_2O_5 for analysis. M. p. 139—140° under evolution of gas.

$C_{12}H_{14}O_3N_2S$ (266.31) Calc. N 10.52 S 12.04
Found » 10.46 » 11.99

Neut.equiv. found 265 (microtitration).

Rotation: $[\alpha]_D^{23} = +85.10°$ ($c = 1.1$; methanol).

Oxidative desulphurization of N-phenylthioacetyl-L-asparagine

To a solution of 266 mg of N-phenylthioacetyl-L-asparagine in 1.00 ml of 1 *N* NaOH was added dropwise with cooling and vigorous stirring 450 mg of 30 % hydrogen peroxide. After half an hour, the reaction mixture was acidified and 220 mg (88 %) of N-phenylacetyl-L-asparagine was collected as colourless crystals. Separated from water as glistening, thin plates. M. p. 189—190°. A non-analyzed preparation with m. p. 180—181° is recorded in the literature [10].

$C_{12}H_{14}O_4N_2$ (250.25) Calc. C 57.58 H 5.64 N 11.20
Found » 57.80 » 5.80 » 11.14

Neut.equiv. found 249 (microtitration).

Rotation: $[\alpha]_D^{23} = +8.95°$ ($c = 1.0$; methanol (supersat.)).

Phenacetylation of L-asparagine

When 2.00 g of L-asparagine was treated with 2.5 g of phenylacetyl chloride in a solution of potassium bicarbonate according to Schotten-Baumann, 1.65 g of N-phenacetyl-L-asparagine was obtained. Recrystallized twice from hot water. M. p. 189°.

Rotation: $[\alpha]_D^{23} = +9.01°$ ($c = 1.0$; methanol).

SUMMARY

Carboxymethyl dithiophenylacetate has been prepared, and its potentialities as a phenylthioacetylating agent towards amines, amino acids and peptides have been demonstrated.

The reactions proceed without racemization.

The microanalyses have been performed in this Laboratory by Mr. A. Grossmann.
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