Quaternary Ammonium Bases in the Tomato Plant

(2-chloroethyl) trimethylammonium chloride (chlorocholine chloride, CCC) and related compounds have marked growth-inhibitory effects when applied to many plants[1–3]. It is therefore of interest to study the effect of the application of these substances upon the metabolism of native choline and choline derivates in the plant, and to consider a possible role of these native substances in the growth process.

By means of paper chromatography, a preliminary investigation of the quaternary ammonium bases of untreated tomato plants has been made. The methods used were developed from those of MAYR and PRESOLY[4], who reported the presence in extracts of tomato and wheat plants of a Dragendorff-reactive substance which they were provisionally unable to distinguish from CCC. As described below, we have now been able to resolve this unknown substance into three components, all distinguishable from CCC.

Chromatograms of aqueous extracts of young tomato plants (var. Bonner Beste) on Whatman No. 1 paper were run in the following solvent systems:

(1) isopropanol-ammonia-water 80:5:15
(2) *n*-butanol-aceticacid-water 4:1: 5
(3) *n*-butanol-ethanol-water 5:1: 4
(4) ethanol-water 8:1

The following colour reagents were used:

(1) Modified Dragendorff reagent: THIESS and REUTHER[5]
(2) Phosphomolybdic acid reagent: CHARGAFF et al.[6] in the modification cited by HAIS and MACEK[7]
(3) Iodine vapour
(4) Iodoplatinate reagent: MERCK[8]
(5) Ammoniummolybdate-perchloric acid reagent: HANES and ISHERWOOD[9].

Three reactive substances, S I, S II, and S III were recognised in the plant extracts, of which S II gave the colour reactions and Rf values of choline. Rf values and colour reactions of these three naturally occurring substances and of known marker compounds are given in Tables I and II.

No evidence could be found of production of the water-soluble bases from phospholipids during the extraction procedure (coldwater extraction of frozen material), but such a production in the brief interval between harvesting and freezing could not be ruled out.

The vacuum-concentrated aqueous extracts were purified by alcohol-precipitation and passage of the alcoholic solution through an aluminium oxide column. Chromatography of the column filtrate yielded one reactive spot, S III, with occasional traces of S I and S II.

When the column filtrate was subjected to paper electrophoresis on Whatman No. 3 MM paper in phosphate buffer pH 6 at 5 mA and 30 V per cm, chromatography of eluates from the region near the starting line yielded mainly substance S III, with small amounts of S II, while S I and S II, but not S III, were present in the cathode region of the electropherogram. When electrophoresis was continued until no Dragendorff-reactive material remained at the starting line, only S I and S II could be recovered, S III having disappeared.

It may be concluded that the main quaternary ammonium base extractable from tomato plants by the methods used is a substance S III, which is not choline, but is readily convertible to choline (S II) and a third substance, S I, during electrophoresis. A close relationship between the substances is suggested by the identity of their colour reactions and by the considerable tailing of

[1] N. E. TOLBERT, J. biol. Chem. *235*, 475 (1960).
[2] N. E. TOLBERT, Plant Physiol. *35*, 380 (1960).
[3] S. H. WITTWER and N. E. TOLBERT, Amer. J. Bot. *47*, 560 (1960).
[4] H. H. MAYR and E. PRESOLY, Planta *57*, 478 (1961).
[5] H. THIES and F. W. REUTHER, Naturwiss. *41*, 230 (1954).
[6] E. CHARGAFF, C. LEVINE, and C. GREENE, J. biol. Chem. *175*, 67 (1948).
[7] I. M. HAIS and K. MACEK, *Handbuch der Papierchromatographie* (Gustav Fischer Verlag, Jena 1960), vol. I, p. 738.
[8] Merck AG, Darmstadt: *Chromatographie*, p. 136.
[9] C. S. HANES and F. A. ISHERWOOD, Nature *164*, 1107 (1949).

Tab. I. Rf values of known quaternary ammonium bases and of those in tomato extracts (see text)

	S I	S II	S III	Choline	Acetylcholine	Phosphorylcholine[a]	CCC
Isopropanol-ammonia-H_2O	0.13	0.26	0.35	0.26	[b]	0.00	0.29
Butanol-acetic acid-H_2O	0.26	0.26	0.28	0.17[c] 0.24	0.30		0.38
Butanol-ethanol-H_2O	0.03	0.15	0.19	0.14	0.18		0.20
Ethanol-H_2O	0.29	0.58	0.59	0.57		0.00	0.61

[a] As calcium salt. [b] Hydrolysed in this solvent system. [c] Gives a double spot in this solvent system.

Tab. II. Colour reactions of chromatogram spots (see text)

	S I	S II	S III	Choline	Acetylcholine	Phosphorylcholine	CCC
Dragendorff	violet	violet	violet	violet	orange-red	orange-red	carmine-red
Phosphomolybdate	blue ++	blue +	blue +++	blue +	blue +	blue tr.	blue +++
Iodine vapour	tr.	tr.	—	tr.	tr.	—	+++
Iodoplatinate	tr.	tr.	tr.	tr.	tr.	—	blue +++
Molybdate-perchloric	0	0	0	0	—	blue	0

0 = no reaction; — = not tested.

spots which occurred on chromatograms, especially those developed in isopropanol-ammonia-water. When eluted from a chromatogram and rechromatographed, S II and S III were stable, but S I was converted to S II during chromatography.

No evidence has so far been obtained that any of the unknown substances is a phosphorylated derivative of choline. With Dragendorff reagent, phosphoryl choline gives a red colour, as do acetyl choline and CCC (as well as other choline derivatives substituted at the hydroxyl position), while the unknown substances all give the violet colour characteristic of choline itself. The ammoniummolybdate-perchloric acid reagent for phosphoric acid esters gives no reaction with the unknowns.

Details of this and of further work will be published elsewhere.

Zusammenfassung. Im Hinblick auf die Untersuchung der Wirkung von Chlorcholinchlorid im Stoffwechsel der Quartärammoniumverbindungen in Pflanzen wurden deren natürlich vorhandene Substanzen in wässrigen Extrakten von Tomatenpflanzen papierchromatographisch in vier Lösungsmitteln untersucht.

In den Extrakten überwog eine Substanz, die sich von Cholin chromatographisch unterscheidet, die aber dieselbe Farbreaktion mit einem modifizierten Dragendorffschen Reagens gibt. Während der Papierelektrophorese bei pH 6 wird diese Substanz in zwei andere umgewandelt. Eine von diesen entspricht chromatographisch Cholin. Keine von den unbekannten Substanzen aber scheint ein phosphoryliertes Derivat von Cholin zu sein.

H. H. Mayr and R. G. Paxton[10]

Biologische Forschungsabteilung der Österreichischen Stickstoffwerke Aktiengesellschaft, Linz (Austria), June 18, 1962.

[10] Present address: Department of Botany, Royal Holloway College, Egham (Surrey, England).

Erycanosid, ein neues Herzglycosid aus *Erysimum canescens* Roth

Im Laufe der Isolierung von Helveticosid und Erysimosid aus den oberirdischen Teilen von *Erysimum canescens* Roth durch Gegenstromverteilung[1] erhielten wir aus den polaren Anteilen vier weitere, vermutlich neue, kristallisierte Herzglycoside. Im System Toluol-*n*-Butanol-Wasser (1:9:10) sind diese durch die R_f-Werte 0,76, 0,62, 0,59 und 0,50 charakterisiert.

Das Glycosid mit Rf-Wert 0,76 wurde Erycanosid genannt; Smp. 249–253° (Isopropanol-Äther); $[\alpha]_D^{23} = +50{,}4 \pm 2°$ (c = 1,0 in Methanol). Erycanosid ($C_{35}H_{52}O_{15}$ (712,76): Ber. C 58,96, H 7,35%; gef. C 58,87, H 7,40%) zeigte im UV-Absorptionsspektrum neben dem Absorptionsmaximum des Butenolid-Ringes bei 217 mμ ($\log \varepsilon = 4{,}20$) ein zweites Maximum bei 302 mμ ($\log \varepsilon = 1{,}50$). Erycanosid bildet eine krist. Hexa-*O*-acetyl-Verbindung ($C_{47}H_{64}O_{21}$ (965,15): Ber. C 58,50, H 6,71%; gef. C 58,92, H 6,74%) Smp. 234–237° (Aceton-Äther); $[\alpha]_D^{27} = +31{,}50 \pm 2°$ (c = 1,0 in Chloroform).

Die saure Hydrolyse von Erycanosid mit 0,1 *n* H_2SO_4 gab als Aglukon Strophanthidin (identifiziert durch IR-Spektrum, Papierchromatogramm, Schmelzpunkt und Mischschmelzpunkt). Aus den wasserlöslichen Anteilen der Hydrolyse wurde durch Chromatographie an Kohle[2] 2-Desoxycellobiose[3] isoliert; $C_{12}H_{22}O_{10}$ (326,18). Ber. C 44,15, H 6,80%; gef. C 44,89, H 7,01%; Smp. 206–214° (Aceton-Äther); $[\alpha]_D^{25} = +30{,}9 \pm 2°$ (c = 1,9 in Pyridin nach 48 h).

Nach Acetylierung der Biose wurde das krist. Hexa-*O*-acetylderivat[4] erhalten, $C_{24}H_{34}O_{16}$ (578,51). Ber. C 49,86, H 5,94%; gef. C 50,57, H 5,94%; Smp. 194–198° (Aceton-Äther); $[\alpha]_D^{23} = -15{,}8 \pm 2°$ (c = 1,0 in Chloroform). Die saure Hydrolyse der Biose unter energischen Bedingungen[5] lieferte 2-Desoxyglucose und Glucose, die durch Papierchromatographie nachgewiesen wurden[6].

Erycanosid kommt somit die Konstitution Strophanthidin-β-2-desoxy-*D*-glucosido-glucosid zu.

Summary. By application of the counter-current method on extracts of *Erysimum canescens* Roth, four new crystalline glycosides have been isolated besides of Helveticoside and Erysimoside. The structure of one of them, named Erycanoside, has been determined as Strophanthidin-β-2-desoxy-*D*-glucosido-glucoside.

Š. Bauer, O. Bauerová, L. Masler und D. Šikl

Abteilung für Biochemie der Saccharide des Chemischen Institutes der Slowakischen Akademie der Wissenschaften, Bratislava (Tschechoslowakei), 27. Juli 1962.

[1] Š. Bauer, Š. Országh, O. Bauerová, J. Mokrý, L. Masler, D. Šikl und J. Tomko, Planta Medica *8*, 145 (1960).
[2] W. J. Whelan und P. J. P. Roberts, J. chem. Soc. *1953*, 1298.
[3] M. Bergmann und W. Breuers, Liebigs Ann. *470*, 38 (1929).
[4] A. M. Gachokidzé, ž. obšč. chim. *16*, 1914 (1946).
[5] H. Kiliani, Ber. dtsch. chem. Ges. *63*, 2866 (1930).
[6] Wir danken Herrn Prof. Dr. T. Reichstein, Institut für organische Chemie der Universität Basel, und Herrn Dr. J. Sicher, Institut für organische Chemie und Biochemie der Tschechoslowakischen Akademie der Wissenschaften, für die Überlassung von Vergleichsproben von 2-Desoxyglucose. – Die UV-Spektren und Elementaranalysen wurden in der Abteilung für physikalische und analytische Chemie unseres Institutes durchgeführt.

The Microbiological Dehydrogenation of 16β-Methyl-5α-Dehydrocortisone

A recent communication from our laboratories[1] reported the synthesis of 16β-methylprednisone, starting from hecogenin and involving about 15 steps. In continuation of this work an attempt was made to substitute the chemical introduction of the double bonds in the positions 1 and 4 of the A ring by microbiological dehydrogenation. A number of reports have been published on similar conversions of 3-oxy pregnanes and androstanes into their corresponding $\Delta_{1,4}$ derivatives using fermentations with fungi[2], actinomycetes[3], and bacteria[4].

[1] G. G. Nathansohn, G. Winters, and E. Testa, Exper. *17*, 448 (1961).
[2] E. Vischer and A. Wettstein, Exper. *9*, 371 (1953).
[3] T. H. Stoudt, W. J. Mc Aleer, M. A. Kozlowski, and V. Marlatt, Arch. Biochem. Biophys. *74*, 280 (1958).
[4] H. R. Levy and P. Talalay, J. Amer. chem. Soc. *79*, 2658 (1957).