This result confirms that cassamic acid has the same carbon skeleton as cassanic acid[9,11,13], proves that the carbonyl group is attached to carbon atom 7[14] and establishes formula IIIa for cassamic acid.

2. *Erythrophlamic acid.* a) *The quaternary carbomethoxy group, the carbon skeleton, and the position of the oxygen functions.* Erythrophlamic acid (IIa) is hydrogenated over a Pd/C-catalyst to the saturated hydroxy keto acid IXa ($C_{21}H_{32}O_6$), m. p. 227–228°C, $[\alpha]_D^{22} = +50°$. By treatment of this saturated hydroxy keto acid with $NaBH_4$, the corresponding dihydroxy acid Xa is obtained ($C_{21}H_{34}O_6$; m.p. 251–252°C, $[\alpha]_D^{22} = +69°$ in methanol). Energetic alkaline hydrolysis of the latter leads to the dihydroxy dicarboxylic acid Xb ($C_{20}H_{32}O_6$; m.p. 283–284°C, $[\alpha]_D^{22} = +37°$ in methanol). The starting material of the energetic alkaline hydrolysis, the dihydroxy acid Xa, is regenerated from the dicarboxylic dihydroxy acid Xb by esterification with diazomethane followed by mild alkaline hydrolysis of the primary carbomethoxy group.

When the dihydroxy dicarboxylic acid Xb is submitted to the same series of reactions as the corresponding compound IVb of the cassamic series (cf. 1c), a dihydroxy acid is obtained ($C_{20}H_{34}O_4$, m.p. 262–265°C, $[\alpha]_D^{21} = 0°$). Comparison with an authentic sample of dihydroxycassanic acid (Xc) from cassaidine[15] showed the two to be identical.

The experiments described above demonstrate the presence of a quaternary carbomethoxy group and determine the positions of the two oxygen atoms in erythrophlamic acid, as well as its carbon skeleton. However, they provide no information on the point of attachment of the quaternary carbomethoxy group, nor do they indicate which of the two hydroxyls of dihydroxycassanic acid (Xc) is present as a keto group in erythrophlamic acid.

b) *The position of the quaternary carbomethoxy group and of the keto and hydroxyl groups.* Dihydroerythrophlamic acid (IXa) is easily oxidized by chromic acid to a diketo acid (VIIIb, $C_{21}H_{30}O_6$, m.p. 203–204°C, $[\alpha]_D^{22} = +13°$). Clemmensen reduction of the latter affords as the main product dihydrocassamic acid (IIIa)[16].

As the keto group in dihydroerythrophlamic acid (IXa) itself is unaffected by Clemmensen reduction, the conversion of the diketo acid (VIIIb) to dihydrocassamic acid (IIIa), together with the preceding one of the dihydroxy acid Xb to dihydroxycassanic acid (Xc) prove structure IIa for erythrophlamic acid itself.

The results of these and related experiments not reported here, as well as their stereochemical implications, will be published in full elsewhere.

We have to thank Dr. T. J. KING for a very generous supply of vouacapenic acid and the *Institut National pour l'Etude Agronomique du Congo Belge* (INEAC) in Bruxelles for providing us with the bark of *Erythrophleum guineense* G. Don. The Zurich group acknowledges with thanks the financial help of CIBA AG. in Basle. V.P.A. is indebted to the CIBA *Followship Trust* for a CIBA Fellowship.

B.J.(London) thanks the *Pharmaceutical Society of Great Britain* and the *Department of Scientific and Industrial Research* for a research studentship.

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Zusammenfassung

Es wird gezeigt, dass Cassamin und Erythrophlamin, zwei kristalline Nebenalkaloide aus *Erythrophleum guineense* G. Don, neben den anderen schon bekannten Sauerstoffunktionen je eine stark gehinderte Carbomethoxy-Gruppe enthalten. Durch Überführung in Verbindungen bekannter Konstitution wird Formel Ib für Cassamin und Formel IIb für Erythrophlamin bewiesen.

[9] L. G. HUMBER and W. I. TAYLOR, J. chem. Soc. *1955*, 1044.

[14] For the position of the oxygen atoms in the cassaine series cf. W. J. GENSLER and G. M. SHERMAN, Chem. & Ind. *1959*, 223; J. Amer. chem. Soc. *81*, 5217 (1959). – R. B. TURNER, E. G. HERZOG, R. B. MORIN, and A. RIEBEL, Tetrahedron Letters No. 2, 7 (1959). – V. P. ARYA and DAVID W. MATHIESON, J. chem. Soc. *1959*, 3623.

[15] L. RUZICKA and G. DALMA, Helv. chim. Acta *23*, 753 (1940).

[16] Cf. the analogous reduction of 3.7-diketocassanic acid to 7-ketocassanic mentioned under 1c.

Inhibitory Action of some Compounds on Staphylococcal Coagulase

The present communication describes data concerning the action of several substances on the *in vitro* activity of staphylococcal free coagulase. Among these were: 11 antibiotics, 6 chemotherapeutics, 3 antiseptics, ingredients of the preserving solution used in the blood banks, 2 nucleic acids and their salts, 5 dextrans and 29 inorganic salts. All substances were crystalline or the best purity available.

Determination of the coagulase titer, coagulase standard, and selection of plasma were the same as previously described[1,2]. Strain J-373 (*Staphylococcus aureus*, strongly coagulase-positive) was grown in Roux bottles containing 100 ml of brain heart infusion broth with the addition of 'ion mixture'[3]. After 18 h incubation, the culture was divided into two parts: one was centrifuged at 5000 r.p.m. for 30 min and the supernatant was used as the source of coagulase; the second part was shaken and added with living cells as the second source of coagulase. Two series of tubes were prepared. Each tube contained 0·5 ml of saline in which dilution of the substance studied were made. Concentrations varied from 0·46 to 1000 γ/ml. To the first series of tubes 0·5 ml of supernatant, and to the second – of living culture, were added. The tubes were shaken and placed in the incubator (37°C) for 1 h. After this time 0·5 ml of citrated rabbit plasma was added to each tube. They were shaken and incubated at 37°C. Readings were made after 2, 6, and 24 h. All procedures were performed in sterile conditions. The third set consisted of the same amount of tubes containing 0·5 ml of saline, 0·5 ml of supernatant, and 0·5 ml of rabbit plasma and served as control showing the actual coagulase titer.

The substances possessing the inhibitory action on staphylococcal free coagulase are listed in the Table.

Antibiotics had a very small effect on coagulase activity in few instances only. Out of 19 antibiotics studied, spiramycin and oleandomycin only in the highest concentration used (1000 γ/ml)—inhibited the clotting. Bacitracin and cycloserine slightly delayed the appearance of the clot. Other antibiotics were completely without effect and clots were obtained promptly, which were as solid as in the control. Isoniazid, and sulfonamides did not also exert a pronounced action on coagulase activity. The exceptions were:

[1] J. JELJASZEWICZ, Acta microbiol. Polon. *7*, 17 (1958).

[2] J. JELJASZEWICZ, Med. Dośw. Mikrobiol. *10*, 287 (1958).

[3] M. TAGER and H. HALES, Yale J. biol. Med. *20*, 41 (1947).

[4] Details about the preserving solution were obtained through the courtesy of Mr. J. MECZYŃSKI from the Poznań Blood Transfusion Station.

[5] K. WŁODARCZAK and J. JELJASZEWICZ, Arch. Immun. Ter. Dośw., in press (1960).

Substances disturbing the *in vitro* coagulase activity

Substance	Minimal concentration causing coagulase inhibition in γ/ml
Sodium ethylmercurithiosalicylate	0·46
Albucid	0·46
Conserving solution	0·46 ml
Nucleic acid	0·46
Desoxyribonucleic acid	0·46
$CuSO_4$	1·85–7·82[a]
Na_3N	7·82–31·25[a]
$K_2Cr_2O_7$	31·25–125·0[a]
$ZnSO_4$	125·0
$CdSO_4$	215·0– 250·0[a]
$MgCl_2$	125·0–1000·0[a]
Phenol	125·0–1000·0[a]
$H_3P/Mo_3O_{10}/_4$	125·0–1000·0[a]
$PtCl_3$	500·0
$BaCl_2$	500·0
$HgCl_2$	500·0
KJ	500·0
$Al_2/SO_4/_3$	500·0
Sulfamethoxypirydazine	500·0
NH_4Cl	500·0–1000·0[a]
$K_4/Fe/CN/_6$	1000·0
Sulfanilamide	1000·0
Oleandomycin	1000·0
Spiramycin	1000·0

[a] In some cases the degrees of inhibition of coagulase in supernatant and living culture–differed.

sulfanilamide, which acted in the highest concentration and sulfamethoxypiradazine, which inhibited clotting also in a rather great quantity (500 γ/ml). The most marked action among antiseptics was that of sodium ethylmercurithiosalicylate ('thiomersalate', 'merthiolate'). This compound even in the lowest quantity (0·46 γ/ml) completely prevented clotting. The action of phenol and rivanol was slightly inhibitory.

The preserving solution used at the blood banks for transfusion purposes (it is mixed in proportions 1:5 with blood and consists of 30 g of sodium citrate, 30 g of glucose, 5 g albucid, 0·03 g rivanol, 22 ml of 50% HCl, and distilled water to 1000 ml, pH = 4·9–5·1)[4] was strongly inhibitory to coagulase. Even 0·46 ml of it prevented the clotting. It is rather interesting because all tests connected with physiological blood clotting were correct.

Nucleic acids also completely inhibited the coagulase action. Dextrans, with the exception of two samples which slightly delayed the clotting process, did not inhibit the coagulase.

Out of 29 inorganic salts, only 17 influenced coagulase at rather higher concentrations [NH_4Cl, $ZnSO_4$, $CdSO_4$, $Al_2(SO_4)_3$, $CuSo_4$, $K_4Fe(CN)_6$, KJ, $HgCl_2$, Na_3N, $BaCl_2$, $MgCl_2$, $K_2Cr_2O_7$, $FeCl_3$, $PtCl_3$, and $H_3P(Mo_3O_{10})_4$]. Some differences between the inhibition of coagulase concerned in the supernatant and living culture were observed in the experiments with salts.

The mechanism of these inhibitions is unknown in relation to the action of coagulase and physiological blood clotting.

Detailed results and a critical review on the problem described will be published later[5].

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Zusammenfassung

Es wurde der Einfluss von 67 Substanzen (Antibiotika, Chemotherapeutika, Sulfonamide, Antiseptika, Dextrane, Nukleinsäuren und Metallsalze) auf die Aktivität der freien Koagulase der Staphylokokken untersucht, worunter 24 als Inhibitoren auftraten.

Glutamic Acid-Alanine Transaminase in Tobacco Leaves Infected with TMV

In one of the former works, active glutamic acid-alanine transaminase and decarboxylase of glutamic acid in leaves of tobacco and other plants was proved[1].

Later on it was found that decarboxylase, in plants inoculated with TMV, increases in intensity[2].

Here the preliminary results are shown dealing with investigation in transamination in tobacco inoculated with tobacco mosaic virus (TMV). The method of investigation was similar to that described in the former account, and therefore only the most important finding are given here.

A sample (2 g) of leaves, picked in the morning, was ground in a mortar together with 5 ml *M*/15 phosphate buffer pH 7·6. The homogenate thus prepared in a quantity of 3 ml was mixed in a test tube with 2 ml glutamic acid (concentration 0·02 *M*) and 2 ml of pyruvic acid of the same concentration. The sample was divided into two parts; one of which was immediately boiled and the other with a delay of 45 min after incubation at 27°C. After boiling, they were filtered and the product was pipetted onto Whatman No. 1 filter paper and chromatographed in 80% phenol.

The activity of transamination was estimated by the increase of alanine. The chromatograms were coloured with ninhydrin (0·5) and the coloured areas which developed were eluted; measurements were made using Pulfrich photometer according to Kretovich[3].

The tested plants was *Nicotiana tabacum* v. White Burley, grown in a glass-house. It was inoculated with TMV and with the use of carborundum, according to the generally accepted rule.

The results are shown in the diagram. It is evident that, on the second day after inoculation, the transamination increases greatly. On the third day, it drops more than 50% in comparison with sound leaves. The activity of glutamic-alanine transaminase remains on that level until the mosaic appears on the leaves. Later on (4 weeks after inoculation) it increases to 75%.

Nowadays, the generally accepted conviction is that transamination is the cardinal process in the synthesis of amino acids and the inhibition of it may be incomprehensible in the case of plants synthetising TMV.

After the inoculation of tobacco with TMV, its leaves become yellowish day by day. It may be the evidence of decreased activity of some metabolic processes. On the other hand, however, intensive virus synthesis and increased activity of some enzymes[4] and processes are to be observed[5].

It is very likely that inhibition of transamination is due to a peculiar change of plant metabolism caused by the synthesis of extraneous proteins.

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[2] M. Gubański, Nature *186*, 657 (1960).
[3] W. L. Kretovich and J. V. Uspenskaya, Biochem. (USSR) *23*, 248 (1958).
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