M/e	$R \cdot O \cdot R^1$									
	9-Phenanthryl 9-Phenanthryl	9-Ph Phenyl	β-Naphthyl β-Naphthyl	β-Naphthyl α-Naphthyl	α-Naphthyl α-Naphthyl	4-biphenyl Phenyl	2-biphenyl Phenyl	β-Naphthyl Phenyl	α-Naphthyl Phenyl	Phenyl *R* Phenyl R^1
370	*100*	—	—	—	—	—	—	—	—	—
369	55·1	—	—	—	—	—	—	—	—	—
368	13·7	—	—	—	—	—	—	—	—	—
342	*18·8*	—	—	—	—	—	—	—	—	—
341	16·5	—	—	—	—	—	—	—	—	—
270	—	*100*	*100*	*100*	*100*	—	—	—	—	—
269	—	—	80·5	77·0	69.4	—	—	—	—	—
268	—	—	24·6	16·9	25·3	—	—	—	—	—
246	—	—	—	—	—	*100*	*100*	—	—	—
245	—	—	—	—	—	64·5	50·3	—	—	—
244	—	—	—	—	—	16·6	30·7	—	—	—
242	—	*12·9*	*25·2*	*28·8*	*18·6*	—	—	—	—	—
241	—	20·6	22·3	17·8	15·7	—	—	—	—	—
220	—	—	—	—	—	—	—	*100*	*100*	—
219	—	—	—	—	—	—	—	61·1	62·5	—
218	—	—	—	—	—	*10·1*	*7·8*	22·3	20·3	—
217	—	—	—	—	—	5·3	8·0	—	—	—
192	—	—	—	—	—	—	—	*23·0*	*10·3*	—
191	—	—	—	—	—	—	—	30·7	19·5	—
177	8·3	63·8	—	—	—	—	—	—	—	—
170	—	—	—	—	—	—	—	—	—	100
169	—	—	—	—	—	—	—	—	—	73·8
168	—	—	—	—	—	—	—	—	—	37·0
165	18·3	35·1	—	—	—	—	—	—	—	—
153	—	—	—	—	—	43·8	38·4[a]	—	—	—
151	—	—	—	—	—	—	—	—	—	—
142	—	—	—	—	—	—	—	—	—	*39·3*
141	—	—	—	—	—	44·4	10·9	—	—	60·7
140	—	—	—	—	—	—	—	—	—	15·5
127	—	—	39·6[a]	38·4[a]	28·4[a]	—	—	15·3[a]	11·7[a]	—
126	—	—	—	19·2	14·8	—	—	—	—	—
117	—	—	—	—	—	—	—	—	—	7·0[b]
115	—	—	—	—	—	12·6	9·5	—	—	17·1
94	—	—	—	—	—	—	—	—	—	6·8
89	—	—	—	—	—	—	—	—	—	6·0
88	—	—	—	—	—	—	—	—	—	—
78	—	—	—	—	—	—	—	—	—	—
77	—	—	—	—	—	9·4	10·2[a]	—	—	72·7[a]
76	—	—	—	—	—	—	—	—	—	—
65	—	—	—	—	—	—	—	—	—	14·5
63	—	—	—	—	—	—	—	—	—	8·5
51	—	—	—	—	—	—	—	—	—	75·2
50	—	—	—	—	—	—	—	—	—	16·2
39	—	—	—	—	—	—	—	—	—	33·3

Masses underlined *100* represent the abundance of the parent molecular ion. Underlined 25.2 the abundance of the parent minus 28 (P-28) ion. [a] This represents the abundance of one or other of the possible aryl ions from the ether. [b] Represents a metastable ion.

o-Phenoxybiphenyl. The same method was used, but light petroleum b. pt. 60°–80°C was used to recrystallise the ether. This gave 0·91 g *o*-phenoxybiphenyl (35%) m.pt. 37°C.

The author wishes to express his thanks to Dr. R. I. REED for advice, encouragement, and valuable discussion while this work was being carried out: also to the Institute of Petroleum (Mass Spectrometry Panel) for a maintenance grant.

J. M. WILSON

Chemistry Department, The University, Glasgow, February 15, 1960.

Zusammenfassung

Es wird über die ionischen Hauptbruchstücke in den Massenspektren von zehn Diarylätherm berichtet. Die Intensitäten können zur Identifizierung dieser Verbindungen dienen.

The Structure of Cassamine and Erythrophlamine[1]

The crystalline alkaloids cassamine ($C_{25}H_{39}O_5N$) and erythrophlamine ($C_{25}H_{39}O_6N$), isolated[2] from *Erythrophleum guineense* G. Don, are known[3] to be β-dimethylamino-ethanol esters of two unsaturated acids named respectively cassamic acid ($C_{21}H_{30}O_5$) and erythrophlamic acid ($C_{21}H_{30}O_6$). Cassamic acid contains one double bond αβ to the carboxyl group, one keto and one methoxyl group. Erythrophlamic acid, in addition to the same functional groups, also carries a hydroxyl group. In both acids the nature of one oxygen atom, the carbon skeleton and the position of the functional groups remained to be determined[4].

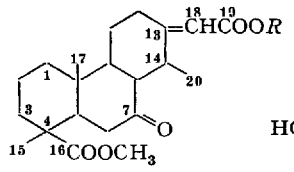
[1] 16th Communication on Erythrophleum Alkaloids. 15th Comm.: B. G. ENGEL, Helv. chim. Acta *42*, 1127 (1959).

[2] B. G. ENGEL and R. TONDEUR, Exper. *4*, 430 (1948); Helv. chim. Acta *32*, 2364 (1949).

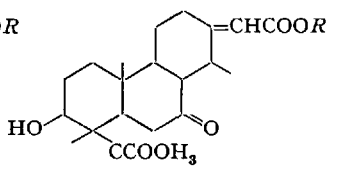
[3] B. G. ENGEL, R. TONDEUR, and L. RUZICKA, Rec. Trav. chim. Pays-Bas *69*, 396 (1950).

[4] The numbering used is that common to steroids and triterpenes.

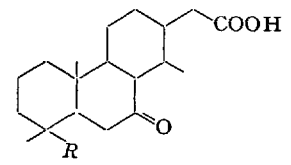
It has now been found that the two acids contain a strongly hindered quaternary carbomethoxy group. Furthermore the following results prove the complete structure of the acids to be Ia for cassamic and IIa for erythrophlamic and consequently Ib and IIb respectively for cassamine and erythrophlamine.



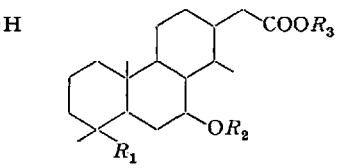
Ia R = H
b R = $CH_2CH_2N(CH_3)_2$



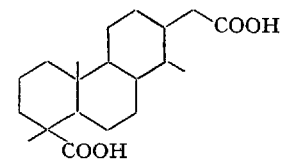
IIa R = H
b R = $CH_2CH_2N(CH_3)_2$



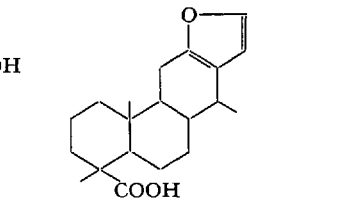
IIIa R = $COOCH_3$
b R = CH_3



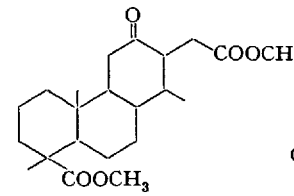
IVa R_1 = $COOCH_3$; R_2=R_3=H
b R_1 = COOH; R_2=R_3=H
c R_1 = COOH; R_2=$COCH_3$; R_3=CH_3
d R_1 = COCl; R_2=$COCH_3$; R_3=CH_3
e R_1 = CHO; R_2=$COCH_3$; R_3=CH_3
f R_1 = CH_3; R_2=R_3=H



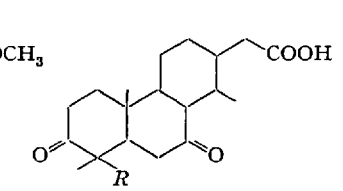
V



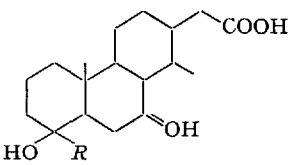
VI



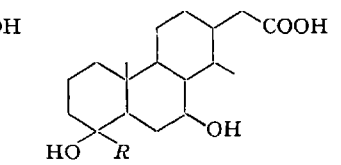
VII



VIIIa R = CH_3
b R = $COOCH_3$



IXa R = $COOCH_3$
b R = CH_3



Xa R = $COOCH_3$
b R = COOH
c R = CH_3

1. *Cassamic acid.* a) *The carbomethoxy group.* Hydrogenation of cassamic acid (Ia) over palladium on charcoal yields dihydrocassamic acid (IIIa, $C_{21}H_{32}O_5$, m. p. 152°C, $[\alpha]_D^{22.5}$ = + 50°C)[6]. Reduction of IIIa with $NaBH_4$ affords the dihydro hydroxy acid IVa ($C_{21}H_{34}O_5$, m. p. 173°C, $[\alpha]_D^{22}$ = + 68°). The latter under energetic alkaline hydrolysis gives rise to the hydroxy dicarboxylic acid IVb ($C_{20}H_{32}O_5$, m. p. 262–263°C, $[\alpha]_D^{21}$ = + 46° in methanol, pK_{MCS}[7] 7, 18; 8,32). That no rearrangement takes place under the conditions of reaction is shown by the reconversion of IVb into the crystalline starting material IVa by esterification with diazomethane followed by mild hydrolysis.

These experiments establish that cassamic acid contains a quaternary carbomethoxy group.

b) *The carbon skeleton and the position of the quaternary carbomethoxy group.* Dihydrocassamic acid (IIIa) under Wolff-Kishner conditions yields the diacid V, concomitant hydrolysis of the quaternary carbomethoxy group taking place. This dicarboxylic acid V ($C_{20}H_{31}O_4$) m.p. 260–261°C and $[\alpha]_D^{20}$ = + 40°, proved identical with a product prepared from vouacapenic acid (VI)[8,9] by the following route. The keto dimethyl ester VII described by KING *et al.*[9], was subjected to thioketal hydrogenolysis followed by vigorous hydrolysis: the same diacid V was obtained.

As the structure of vouacapenic acid has been determined[8,9], the above correlation provides definite proof for the carbon skeleton of dihydro cassamic acid and for the position of the quaternary carbomethoxy group as depicted in formula IIIa. That the acid side chain at C_{13} is unsaturated in cassamic acid itself Ia is shown by the isolation of oxalic acid on ozonolysis.

c) *The position of the carbonyl group.* In another series of reactions the quaternary carboxyl group at C_4 in the hydroxy dicarboxylic acid IVb was converted to a methyl group through the steps: hydroxy dicarboxylic acid IVb → primary monomethylester acetate IVc → acid chloride IVd →[10] aldehydo acetoxy methyl ester IVe → hydroxy acid IVf. The final product of this conversion possessed the empirical formula $C_{20}H_{34}O_3$, m. p. 226°C and $[\alpha]_D^{22}$ = + 42°, and proved to be identical in all respects with an authentic sample of 7-hydroxycassanic acid (IVf). The latter was obtained from 3,7-diketocassanic acid[11] (VIIIa) by Clemmensen reduction followed by treatment of the resulting 7-ketocassanic acid (IIIb) with $NaBH_4$[12].

[5] Optical rotations are in ethanol unless otherwise stated.

[6] Satisfactory microanalyses have been obtained for all compounds mentioned. Spectral properties are in accord with the stated structures.

[7] The notation pK_{MCS}^{*} denotes the negative logarithm of the apparent dissociation constant in 80% methylcellosolve/water mixture (w/w) as determined by the method of W. SIMON, Helv. chim. Acta *41*, 1835 (1958). The determinations were carried out by Dr. W. SIMON, ETH, Zurich, whom we wish to thank for his kind co-operation.

[8] F. E. KING, D. H. GODSON, and T. J. KING, J. chem. Soc. *1955*, 1117.

[9] F. E. KING, T. J. KING, and J. M. UPRICHARD, J. chem. Soc. *1958*, 3428.

[10] Selective reduction with $LiAl(OBu^t)_3H$, cf. H. C. BROWN and R. F. MCFARLIN, J. Amer. chem. Soc. *80*, 5372 (1958).

[11] L. RUZICKA, G. DALMA, and W. E. SCOTT, Helv. chim. Acta *24*, 184E (1941).

[12] That only the 3-keto group of 3,7-diketocassanic acid (VIIIa) is reduced follows from the fact that the product of the Clemmensen reduction (IIIb) differs from 3-ketocassanic acid obtained by A. RONCO, Diss. ETH, Zurich (1945) through Wolff-Kishner reduction of dihydrocassaic acid (IXb) and subsequent chromic acid oxidation of the original 3-hydrocassanic acid. The latter is also found to be different from the hydroxy acid IIIb.

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.

D. W. MATHIESON, B. JAQUES, G. T. CHAPMAN*, V. P. ARYA, and B. G. ENGEL**

** School of Pharmacy, London. ** Organisch-chemisches Laboratorium der Eidg. Technischen Hochschule, Zürich, July 20, 1960.*

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.

[13] 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: 1[1] 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).