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ELUCIDATION OF THE STRUCTURE OF BONGKREKIC ACID—II*

CHEMICAL STRUCTURE OF BONGKREKIC ACID AND STUDY OF THE UV, IR, NMR AND MASS SPECTRA

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Abstract—A structural study of the toxic antibiotic bongkrekic acid (BA) has been performed. From chemical and spectroscopic evidence obtained for the acid and the totally reduced acid (HBA) we propose the following formula:
8-{2-(2-carboxy-5-methylcyclopent-2-en-1-ylidene)ethyl}-6-methoxy-2,5-dimethylhexadeca-2,4,9,12(13)-tetraenedoic acid (XXV).

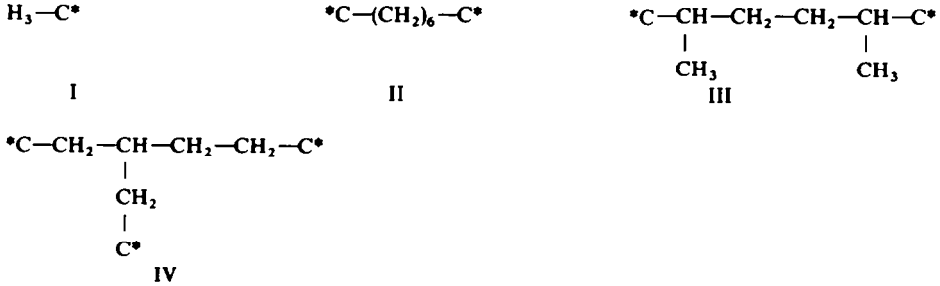
INTRODUCTION

THE CHEMICAL structure of the toxic antibiotic bongkrekic acid (BA), produced by *Pseudomonas cocovenenans* on partially defatted coconut, is studied. In a previous report[1] we described its isolation and purification and showed that BA is a branched unsaturated tricarboxylic acid ($C_{28}H_{38}O_7$). It contains two pairs of conjugated double bonds—both conjugated with a carboxylic group—and two isolated double bonds. The presence of three Me groups, one OMe group and a ring system was demonstrated.

Chromic acid oxidation of hydrobongkrekic acid

By catalytic hydrogenation with 10% Pd on charcoal BA was converted[1] to the very stable hydrobongkrekic acid (HBA) ($C_{28}H_{50}O_7$). For the investigation of the carbonskeleton of bongkrekic acid HBA seems to be the obvious product[2, 3, 4, 5, 6]. Cleavage of HBA with chromic acid provided a mixture of acids, identified by GLC/MS (Table 1).

From the products the presence in HBA of the following structure fragments (I, II, III, IV) can be concluded.



* Part of thesis of G. W. M. Lijmbach, Delft, 1969.

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TABLE 1

Peak nr.	Mass	Gross formula	Compund
1	146	$C_6H_{10}O_4$	$H_3COOC-CH_2-CH_2-COOCH_3$
2	160	$C_7H_{12}O_4$	$H_3COOC-CH_2-CH(CH_3)-COOCH_3$
3	160	$C_7H_{12}O_4$	$H_3COOC-CH_2-CH_2-CH_2-COOCH_3$
4	174	$C_8H_{14}O_4$	$H_3COOC-CH(CH_3)-CH_2-CH_2-COOCH_3$
5	174	$C_8H_{14}O_4$	$H_3COOC-CH_2-CH_2-CH_2-CH_2-COOCH_3$
6	202	$C_{10}H_{18}O_4$	$H_3COOC-CH(CH_3)-CH_2-CH_2-CH(CH_3)-COOCH_3$
7	188	$C_9H_{16}O_4$	$H_3COOC-CH_2-CH_2-CH_2-CH_2-CH_2-COOCH_3$
8	218	$C_9H_{14}O_6$	$H_3COOC-CH_2-CH(COOCH_3)-CH_2-COOCH_3$
9	202	$C_{10}H_{18}O_4$	$H_3COOC-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COOCH_3$
10	232	$C_{10}H_{16}O_6$	$H_3COOC-CH_2-CH(CH_2COOCH_3)-CH_2-COOCH_3$
11	246	$C_{11}H_{18}O_6$	$H_3COOC-CH_2-CH(CH_2-COOCH_3)-CH_2-COOCH_3$

As AcOH is the largest monocarboxylic acid in the reaction mixture, there are in HBA no saturated terminal alkane groups larger than Me. The NMR spectrum of HBA shows the presence of three Me groups

2x—CH—
|
CH_3 $\delta = 0{\cdot}87$ ppm (6H, d, $J = 6{\cdot}0$ Hz).

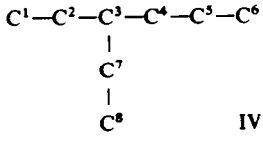
—CH—COOH
|
CH_3 $\delta = 1{\cdot}13$ ppm (3H, d, $J = 7{\cdot}0$ Hz).

Suberic acid is the largest dicarboxylic acid in the oxidation mixture, thus an unbranched chain of at least six carbon atoms must be present in HBA (fragment II).

An unbranched chain of seven or eight CH_2— groups may also occur, because on chromic acid oxidation either two carboxyl groups are formed or only one carboxyl group is formed, while in the latter case the other carboxyl group must already be present in the molecule (HBA).

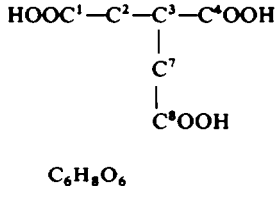
The presence of 2,5 dimethyladipic acid in the oxidation mixture indicated the presence of fragment III in HBA. The *C-atoms are located in such a way that they can form carboxyl groups on chromic acid oxidation. By oxidation of III methylsuccinic acid can also be formed.

The formation of the three tricarboxylic acids can be explained by the assumption that fragment IV is present in HBA:

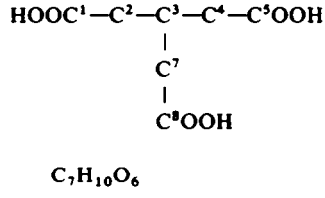


IV

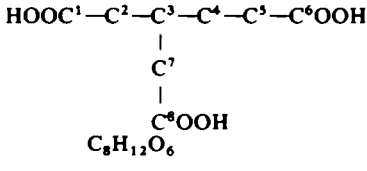
C^1 and C^8 must be placed in HBA in such a way that they will preferably be oxidized to carboxyl groups. C^1 (and/or C^8) may already be present in HBA as a carboxyl group. From fragment IV the three tricarboxylic acids found can be formed as follows:



$C_6H_8O_6$



$C_7H_{10}O_6$



$C_8H_{12}O_6$

Determination of the Location of the methoxyl group in hydrobongkrekic acid

In our previous report[1] we showed the presence of one MeO group in HBA. By treatment with acetic acid anhydride and *p*-TsOH HBA could be demethoxylated and dehydrated[7]. The double bond, thus formed, was ozonized and the products of the ozonolysis were investigated (Fig. 1).

In the destillate the following were identified (GLC/MS) (1) methylsuccinic acid, (2) 2-methyl-4-acetyl butyric acid, (3) 2,5-dimethyladipic acid, (4) 2,5-dimethylpimilic acid

If we assume structure fragment V to be present in HBA the formation of these four compounds can be explained.

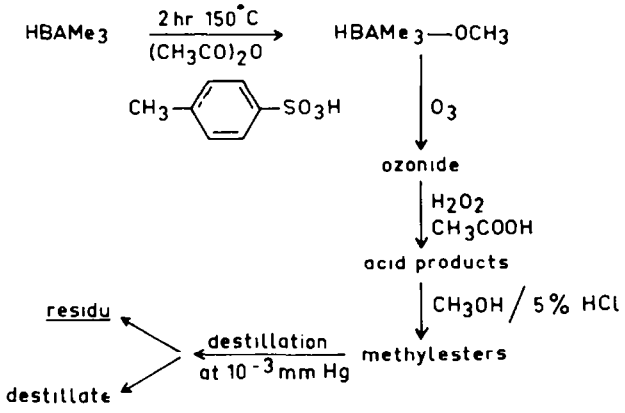
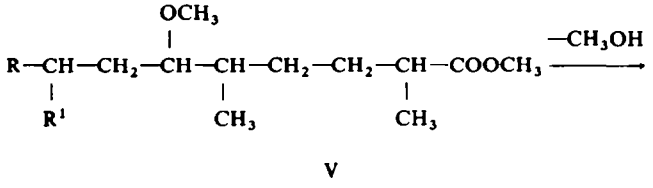
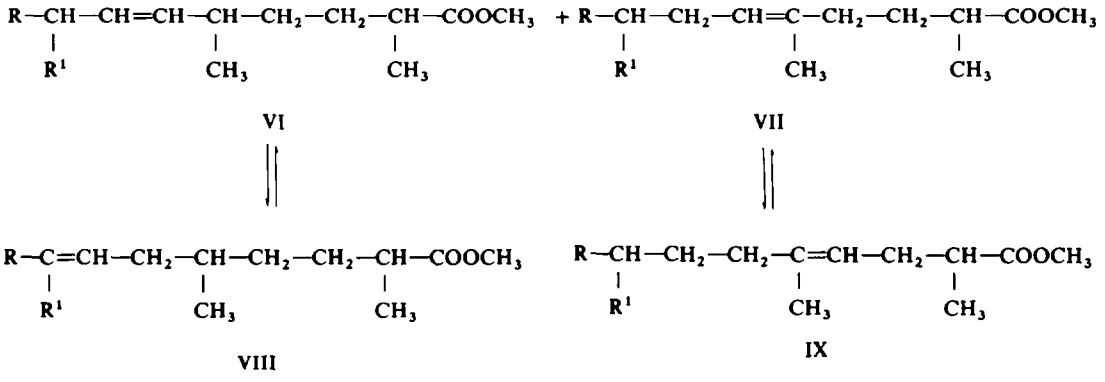


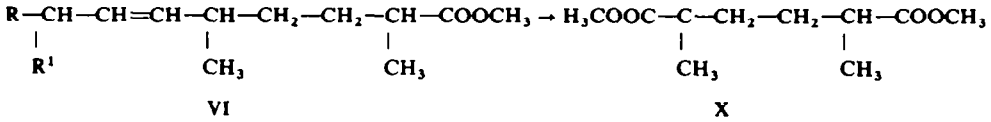
FIG 1. Demethoxylation of HBA.

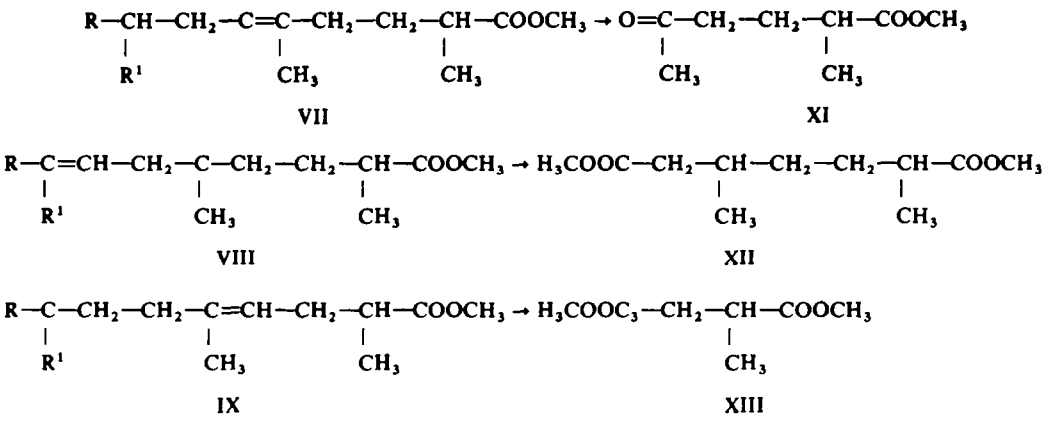


V



Demethoxylation leads to the formation of a double bond in two possible ways and they can isomerize under the influence of *p*-TsOH.[8] From ozonolysis of VI, VII, VIII and IX, followed by esterification with CH_3OH/5% HCl/10%$(CH_3)_2CO_3$ we obtain X, XI, XII and XIII.





The presence of structure fragment V in $HBAMe_3$ and its cleavage pattern can be concluded from the products found and their relative quantities. A double bond will preferably be formed with the tertiary C-atom (VI). The main ozonolysis product must thus be: 2-methyl-4-acetyl butyric acid methylester. By isomerisation IX is formed from VII, and so we may expect a reasonable amount of methyl methy succinate after ozonolysis. VI will be formed less preferentially. The quantity of ozonolysis product X (dimethyl 2,5-dimethy adipate) is indeed less than the quantity of the said two products. Ozonolysis product XII from isomerization product VIII is also clearly present in the reaction mixture. Structure fragment V appears to be a very plausible fragment of $HBAMe_3$. In fact it is an enlargement of fragment III found from the chromic acid oxidation of HBA.

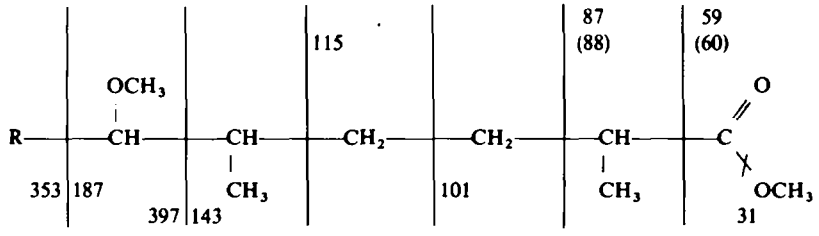


FIG 2.

The presence of structure fragment V was strongly supported by the following peaks and metastable peaks in the MS of $HBAMe_3$ (Fig. 2).

(a) $m/e = 187\ C_{10}H_{19}O_3$

(b) $m/e = 155\ C_{10}H_{19}O_3(187) — CH_4O(32)\ C_9H_{15}O_2(155)$(base peak) $M^* = 128{\cdot}5$
$m/e = 123\ C_{19}H_{15}O_2(155) — CH_4O(32)\ C_8H_{11}O(123)\ M^* = 97{\cdot}6$

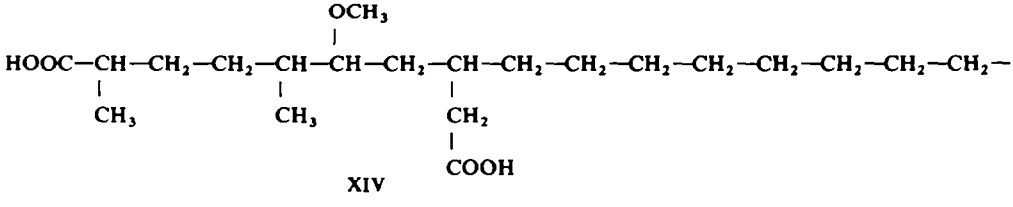
(c) $m/e = 115\ C_6H_{11}O_2(115) — CH_4O(32)\ C_5H_7O(83)\ M^* = 59{\cdot}9$
$m/e = 143\ C_8H_{15}O_2(143) — CH_4O(32)\ C_7H_{11}O(111)\ M^* = 86{\cdot}2$
$m/e = 397\ C_{23}H_{41}O_5\ (P - 143)$
$m/e = 353\ C_{21}H_{37}O_4\ (P - 187)$
$m/e = 88\ \ C_4H_8O_2$
$m/e = 101\ C_5H_9O_2$ (lit. 22).

Still more evidence for the presence of structure fragment V in HBA was found by chromic acid oxidation of the "residue" (Fig. 1). This resulted in the same products as we obtained from the chromic acid oxidation of HBA itself, except for methylsuccinic acid and 2,5-dimethyladipic acid (Table 2).

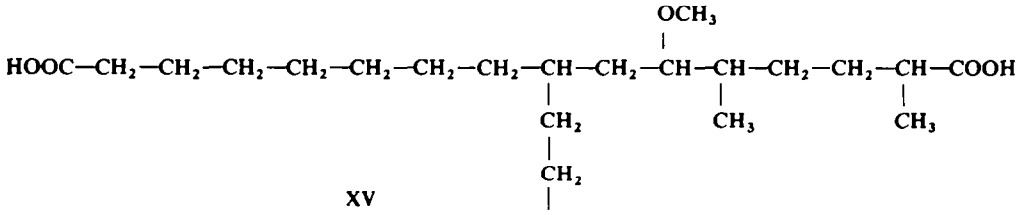
TABLE 2. CHROMIC ACID OXIDATION OF HBA

HBA	Residue from destillation after demethoxylation and ozonolysis of HBA
$HOOC-(CH_2)_2-COOH$	$HOOC-(CH_2)_2-COOH$
$HOOC-CH(CH_3)-CH_2-COOH$	—
$HOOC-(CH_2)_3-COOH$	$HOOC-(CH_2)_3-COOH$
$HOOC-CH(CH_3)-CH_2-CH_2-COOH$	$HOOC-CH(CH_3)-CH_2-CH_2-COOH$
$HOOC-(CH_2)_4-COOH$	$HOOC-(CH_2)_4-COOH$
$HOOC-CH(CH_3)-CH_2-CH_2-CH(CH_3)-COOH$	—
$HOOC-(CH_2)_5-COOH$	$HOOC-(CH_2)_5-COOH$
$HOOC-CH_2-CH(COOH)-CH_2-COOH$	$HOOC-CH_2-CH(COOH)-CH_2-COOH$
$HOOC-(CH_2)_6-COOH$	$HOOC-(CH_2)_6-COOH$
$HOOC-CH_2-CH(CH_2COOH)-CH_2-COOH$	*Less* $HOOC-CH_2-CH(CH_2COOH)-CH_2-COOH$
$HOOC-CH_2-CH(CH_2COOH)-CH_2-CH_2-COOH$	

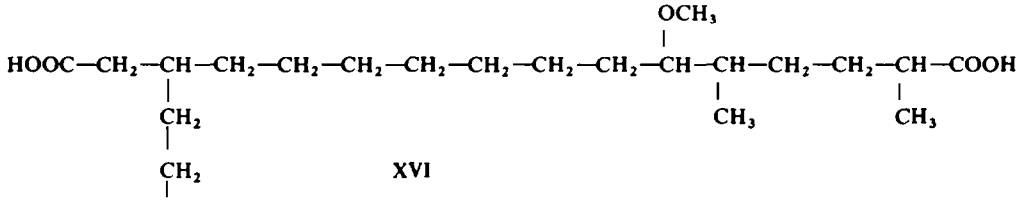
The chromic acid oxidation experiments showed that the structure fragments I, II, IV and V are present in HBA. Thus giving three possible structure fragments for HBA.



XIV

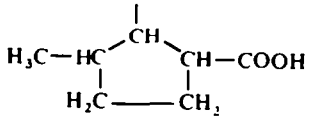


XV



XVI

Leaving 7 C-atoms to determine. One of them must be a carboxyl group. The remaining six C-atoms must deliver a ring system, as has been shown in our previous report[1]. At chromic acid oxidation this fragment must yield 2-methylglutaric acid (Table 2). Assumption of fragment XVII enables us to explain these data. As at oxidation the tertiary C-atoms are preferentially attacked 2-methylglutaric acid and



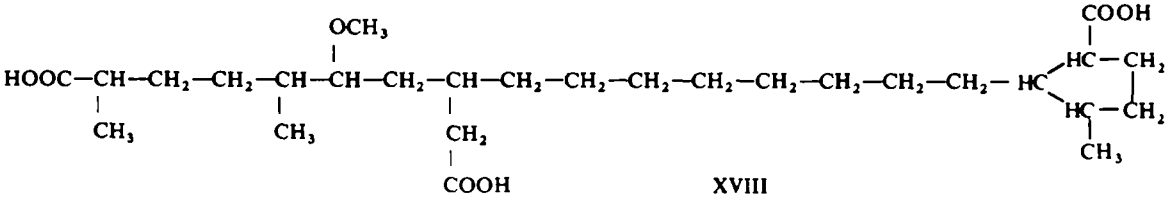
XVII

succinic acid can be expected as a reaction product. Moreover from the products of oxidative ozonolysis of BA the presence of fragment XVII is quite plausible. More evidence for fragment XVII has been obtained from the MS of $HBAMe_3$ (Fig. 8). Cleavage of the ring fragment $C_8H_{13}O_2$(141) from the parent results in fragment $C_{23}H_{13}O_5$(399). Both fragments (m/e = 141 and 399) are present in the MS as well as the expected fragments which are the result of further fragmentation and its additional diffuse peaks:

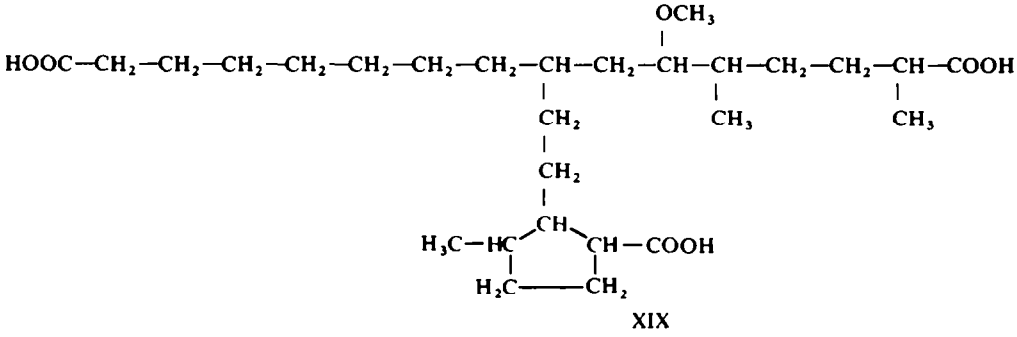
(a) m/e = 399 $C_{23}H_{43}O_5$ (Parent—ringfragment).
m/e = 367 $C_{23}H_{43}O_5$(399)—CH_4O(32) → $C_{22}H_{39}O_4$(367) M* = 337·6
m/e = 335 $C_{22}H_{39}O_4$(367)—CH_4O(32) → $C_{21}H_{35}O_3$(335) M* = 305·8
m/e = 303 $C_{21}H_{35}O_3$(335)—CH_4O(32) → $C_{20}H_{31}O_3$(303) M* = 274·1

(b) m/e = 141 $V_8H_{13}O_2$ (ringfragment)
m/e = 109 $C_8H_{13}O_2$(141)—CH_4O(32) → C_7H_9O(109) M* = 84·3
m/e = 126 $C_8H_{13}O_2$(141)—CH_3(15) → $C_7H_{10}O_2$(126)
m/e = 81 $C_8H_{13}O_2$(141)—$C_2H_4O_2$(60) → C_6H_9(81)
m/e = 82 $C_8H_{13}O_2$(141)—$C_2H_3O_2$(59) → C_6H_{10}(82)

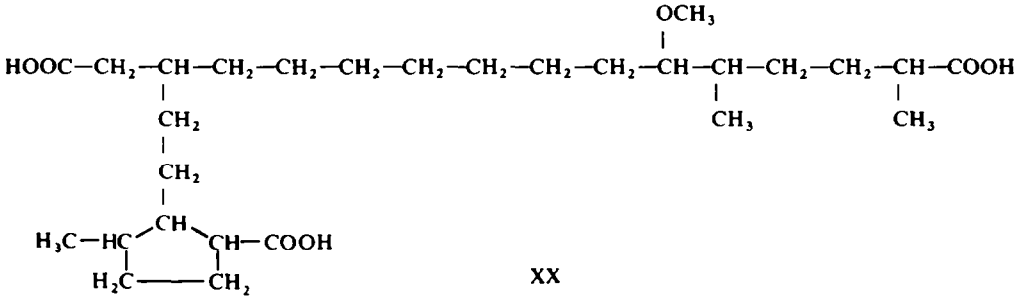
Combination of fragment XVII with the three fragments XIV, XV and XVI results in three possible formulae for HBA (formulae XVIII, XIX and XX).



XVIII



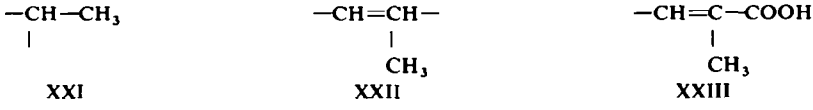
XIX



XX

Determination of the positions of the double bonds in bongkrekic acid

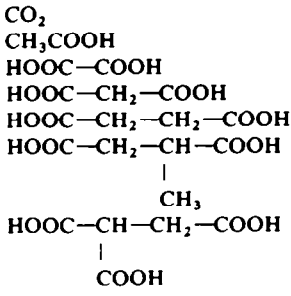
In the previous report[1] the presence of two isolated double bonds and two pairs of conjugated double bonds—both conjugated with a carboxyl group— in BA could be demonstrated. Fragments XXI, XXII and XXIII were shown to be present as well.



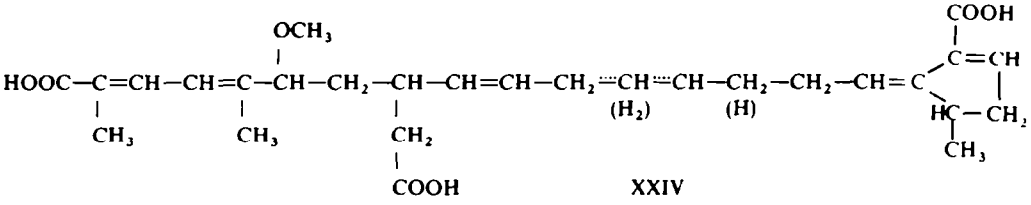
XXI XXII XXIII

Cleavage of the double bonds in BA by ozonolysis[9] resulted in the products that are listed in Table 3.

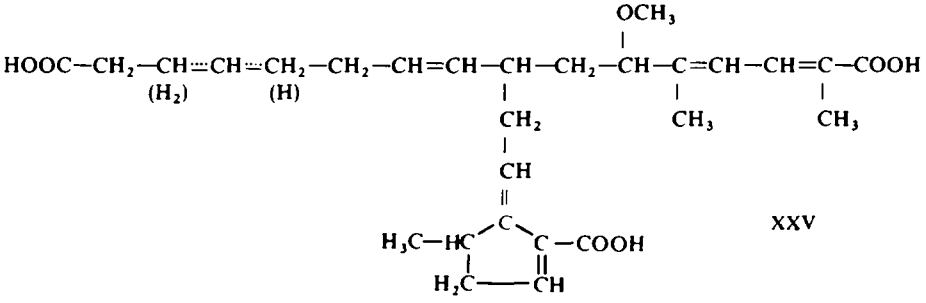
TABLE 3. OXIDATIVE OZONOLYSIS OF BA



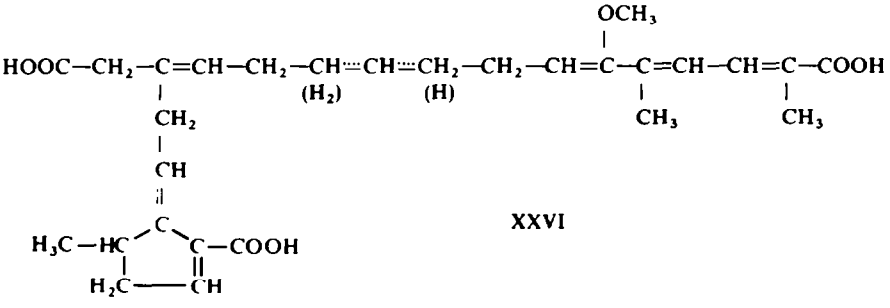
When the six double bonds of BA are placed in the three possible structures of HBA (XVIII, XIX and XX) in such a way that the products listed in Table 3 can be expected on oxidation, formulae XXIV, XXV and XXVI for BA are the result.



XXIV

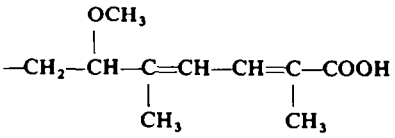


XXV



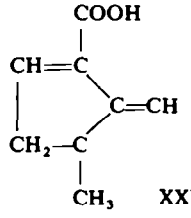
XXVI

Structure fragment XXVII gives on ozonolysis oxalic acid and pyruvic acid, which can decarboxylate into CO_2 and AcOH

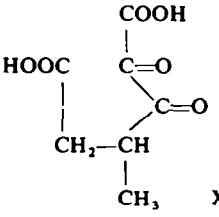


XXVII

Oxidation of fragment XXVIII gives XXIX, which decomposes in methylsuccinic acid and CO_2.

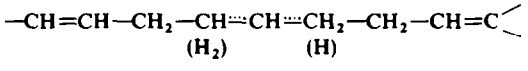


XXVIII

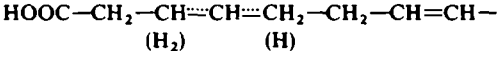


XXIX

In formula XXIV fragment XXX delivers succinic acid and malonic acid, as in formula XXV these two acids can be formed from fragment XXXI.

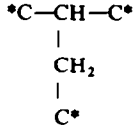


XXX



XXXI

The formation of 2-carboxyl-succinic acid at ozonolysis of BA shows the presence of fragment XXXII in BA (see fragment IV in HBA).



XXXII

Formula XXVI can not be correct, because:

(1) Three conjugated double bonds conjugated with a carboxyl group should give an UV-absorption maximum of about 300 mμ. (BA, maxima at 239 and 263 mμ).
(2) A double bond adjacent to the MeO group should give a higher δ-value in the NMR-spectrum than $\delta = 3{\cdot}20$ ppm, found for BA.
(3) Ozonolysis of product XXVI should give CO_2, AcOH, oxalic acid, malonic acid, succinic acid, methylsuccinic acid and α-keto-glutaric acid. No 2-carboxyl-succinic acid can be formed.

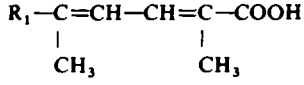
Leaving XXIV and XXV, in which the two possible positions of one of the isolated double bonds are shown by dotted lines.

UV Spectra of bongkrekic acid and hydrobongkrekic acid

The ammonium salt of BA in water absorbs strongly in the UV region: two maxima at 239 mμ (ε = 40,600) and 263 mμ (ε = 40,600) and a minimum at 250 mμ (ε = 37,600). A methanolic solution of BA shows two absorption maxima at 237 mμ (ε = 32,000) and 267 mμ (ε = 36,700) and a minimum at 249 mμ (ε = 28,000). As HBA shows not UV or visible absorption.

Using the Woodward rules[10, 11, 12, 13] we calculated two maxima for the two conjugated systems in BA, suggested in formulae XXIV and XXV.

A

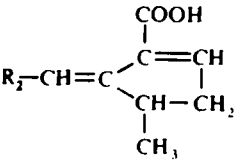


λ_{max} = 214 (butadiene) + 15 (3x alkylrest) + 30 (COOH conjugated with double bonds) = 259 mμ

or λ_{max} = 259 (sorbic acid) + 10 (2x alkylrest) = 269 mμ

Found: λ_{max} = 267 mμ.

B



At the calculation of λ_{max} for this fragment we must consider the phenomenon of "cross conjugation"[12, 14, 15]. λ_{max} = 214 (butadiene) + 10 (2x ringrest) + 5 (alkylrest) + 5 (COOH in "cross conjugation") = 234 mμ.

Found: λ_{max} = 237 mμ.

From the data obtained we may conclude that the UV of BA is in complete agreement with the suggested formulae XXIV and XXV.

IR-Spectra of bongkrekic acid and hydrobongkrekic acid

From the IR-spectra (Fig. 3) we may conclude the absence of aromatic ring systems, OH—, keto— and acetylenic groups and a cyclopropane ring system in BA. The presence of an aliphatic ether (1106 cm^{-1}), methylester groups (1740 cm^{-1} and 1710 cm^{-1} in BA and 1740 cm^{-1} in HBA) and conjugated double bonds (1634 cm^{-1} and 1614 cm^{-1}) are significant.

NMR spectra of bongkrekic acid and hydrobongkrekic acid[19, 23, 24, 25, 26]

The NMR spectra are shown in Figs. 4, 5 and 6.

The NMR spectrum of $BAMe_3$ shows three ester resonances (δ = 3·67; 3·70 and 3·75 ppm). However, in the NMR spectrum of $HBAMe_3$ only *one* ester peak with an intensity of nine protons could be determined (δ = 3·67 ppm). So the differences in ester resonance in $BAMe_3$ are caused by a different conjugation of two ester groups with double bonds.

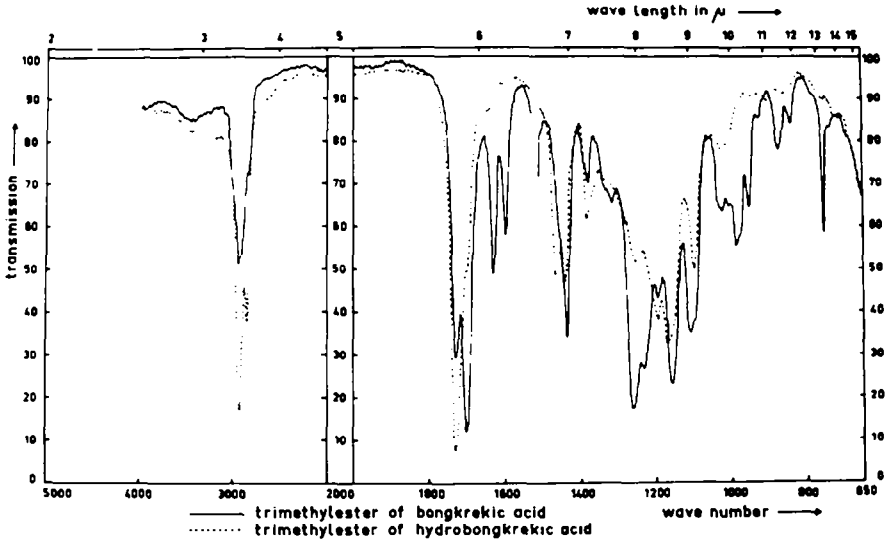


FIG 3a. IR-spectrum of $BAMe_3$ and $HBAMe_3$ (Unicam SP-200)

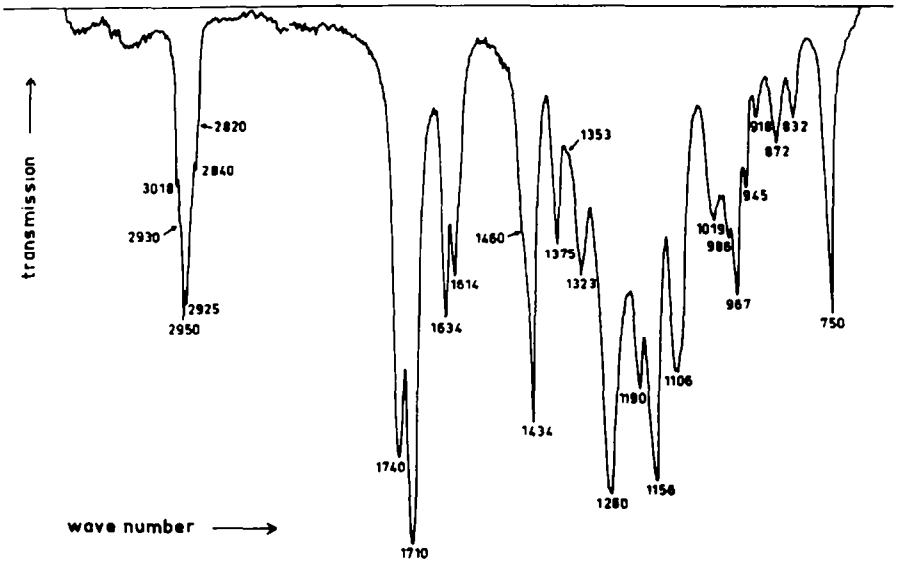


FIG 3b. IR-spectrum of $BAMe_3$ (Hilger-Infrascan)

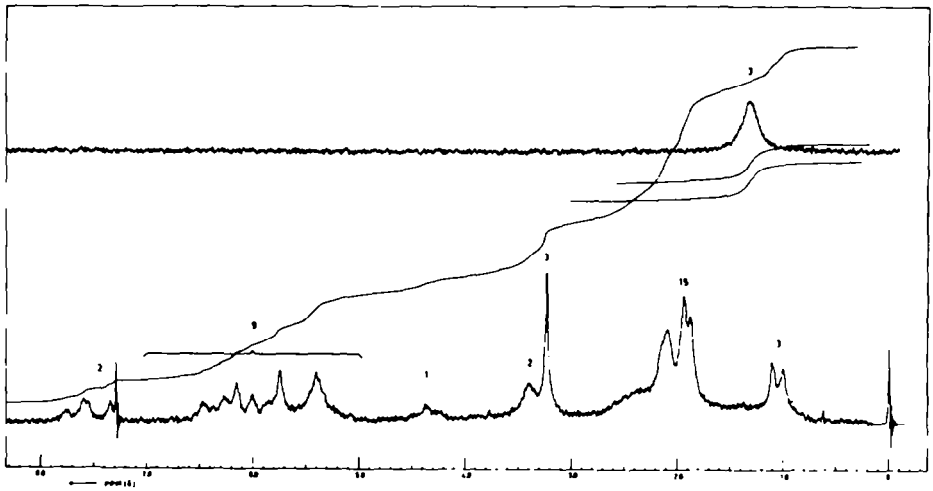


FIG 4. NMR-spectrum of BA (60 MC)

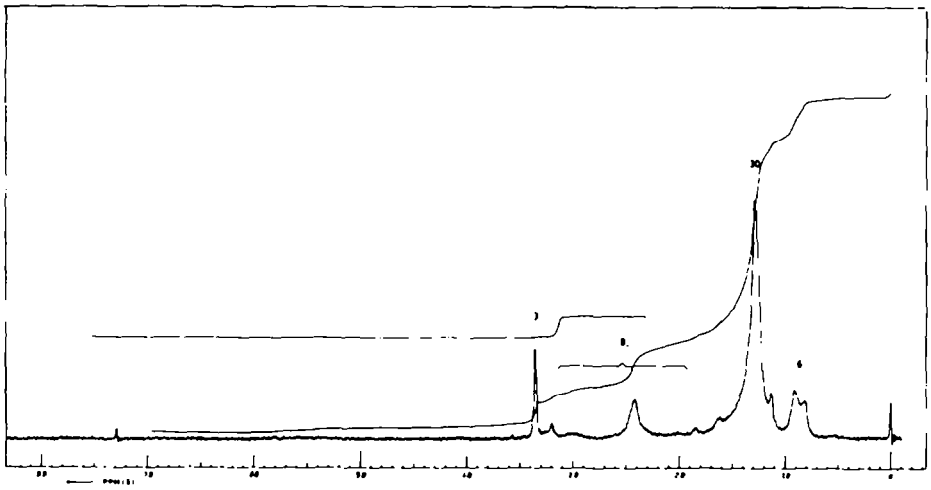


FIG 5. NMR-spectrum of HBA (60 MC)

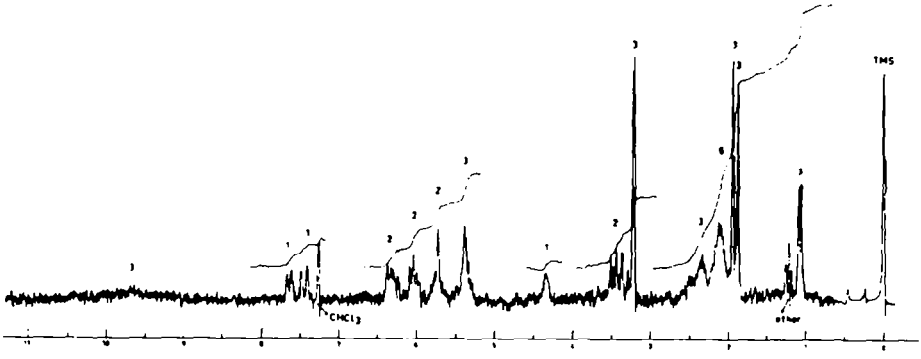


FIG 6. NMR-spectrum of BA (220 MC)

TABLE 4

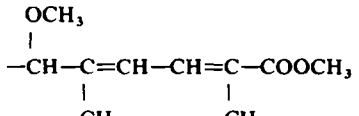
	δ in ppm			Int.	
BA	1·08	d	J = 6Hz	3H	$-CH(CH_3)-$
	1·88	s		3H	$-CH=C(CH_3)-$
	1·95	s		3H	$-CH=C(CH_3)-COOH$
	3·22	s		3H	$-OCH_3$
HBA	0·87	d	J = 6 Hz	6H	2x $-CH(CH_3)-$
	1·13	d	J = 6 Hz	3H	$-CH(CH_3)-COOH$
	3·37	s		3H	$-OCH_3$

The NMR spectra of BA and HBA (Figs. 4, 5 and 6) showed that the presence of three Me groups and one OMe group, was as indicated in formulae XXV and XIX, respectively (Table 4). The difference in OMel resonance of the NMR spectra of BA and HBA may be due to double bond proximity.

Mass spectra of the trimethylesters of bongkrekic acid and hydrobongkrekic acid[19, 20, 21]

Mass spectrum of $BAMe_3$

The fragmentation results in a great number of small fragments, caused by the highly unsaturated character of BA. From the parent peak m/e = 528·3083 we obtained $C_{31}H_{44}O_7$. The base peak (m/e = 183·1017 $C_{10}H_{15}O_3$) proves the presence of



XXXIII ($C_{10}H_{15}O_3$)

fragment XXXIII in $BAMe_3$. The expected fragmentation peaks for this fragment, as well as the additional diffuse peaks were found in the mass spectrum of $BAMe_3$:

$C_{10}H_{15}O_3(183) - CH_4O(32) \rightarrow C_9H_{11}O_2(151)$ $M^* = 124·6$

$C_9H_{11}O_2(151) - CH_4O(32) \rightarrow C_8H_7O(119)$ $M^* = 93·7$

Mass spectrum of $HBAMe_3$

From the MS of $HBAMe_3$ (Fig. 8) we assigned formula XXV as the structural formula formula of BA. Using the element-map technique we know the exact mass and gross formula of each fragment. The presence of structure fragments V and XVII in HBA can be explained clearly from the MS.

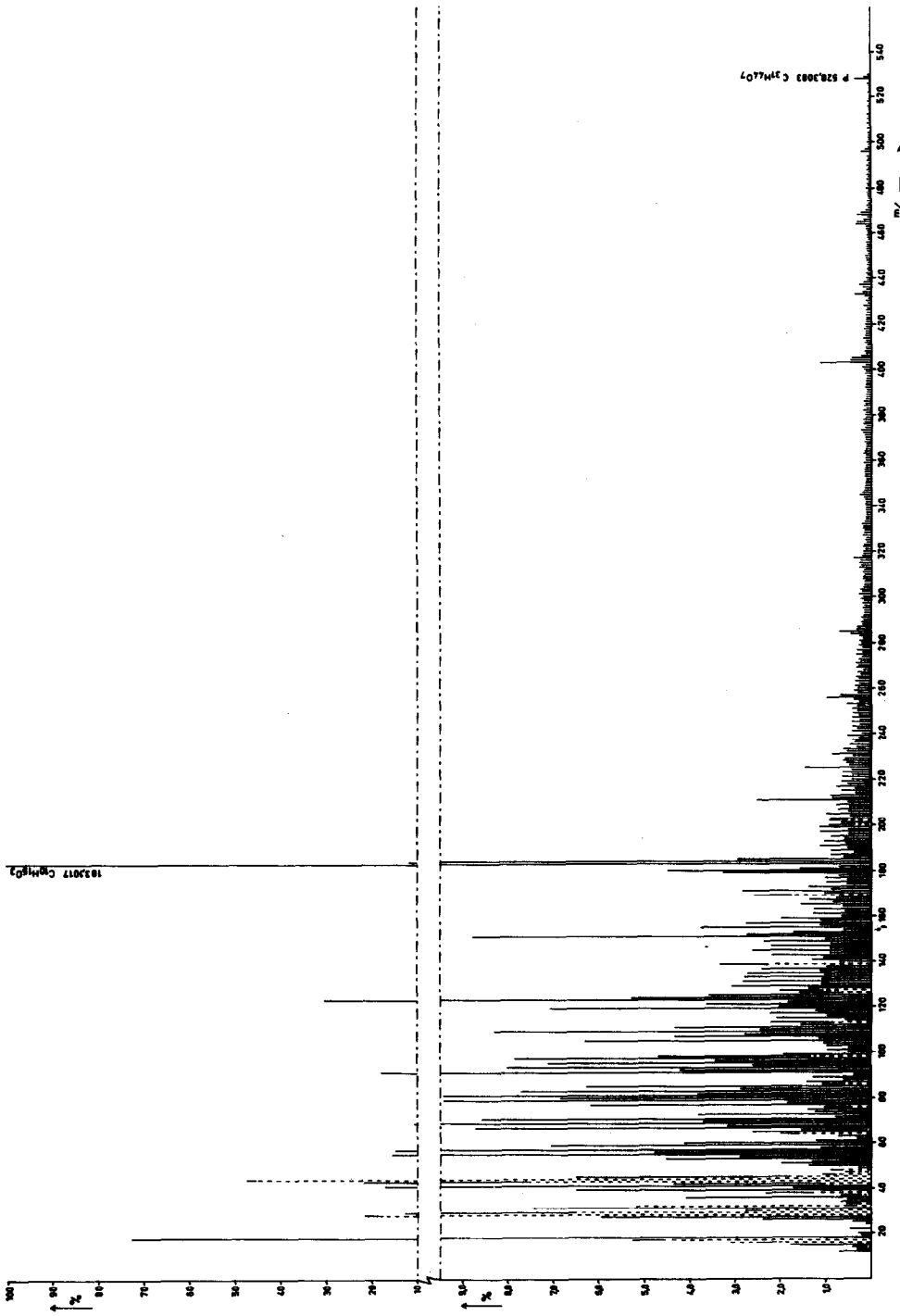


FIG 7. Mass spectrum of $BAMe_3$

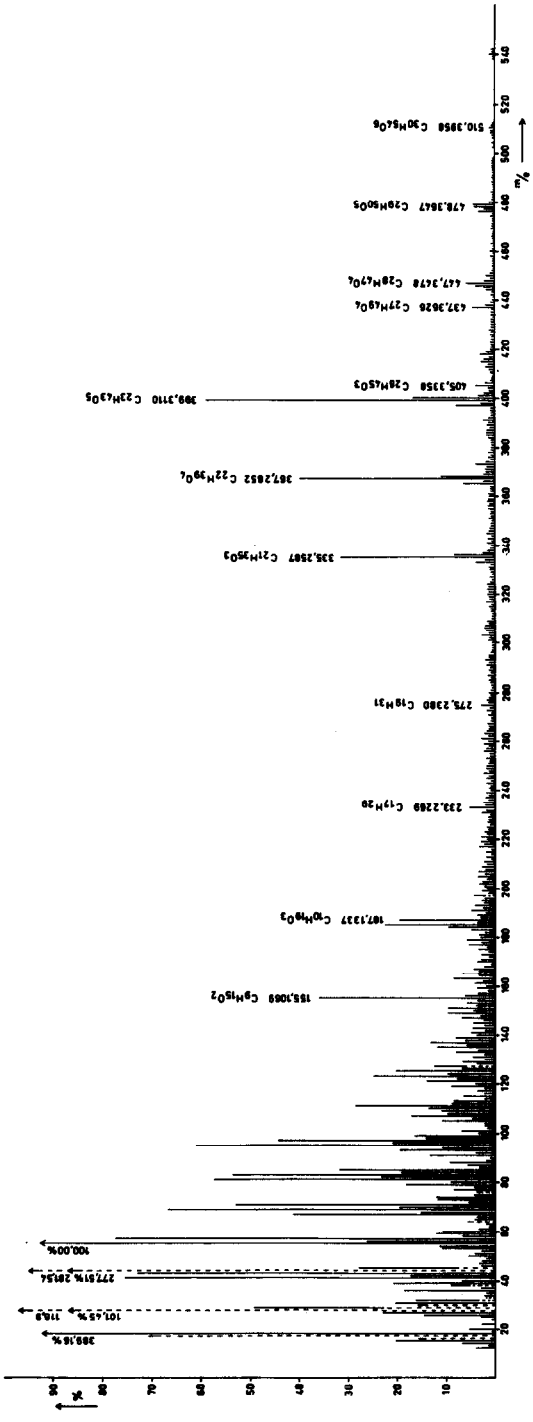
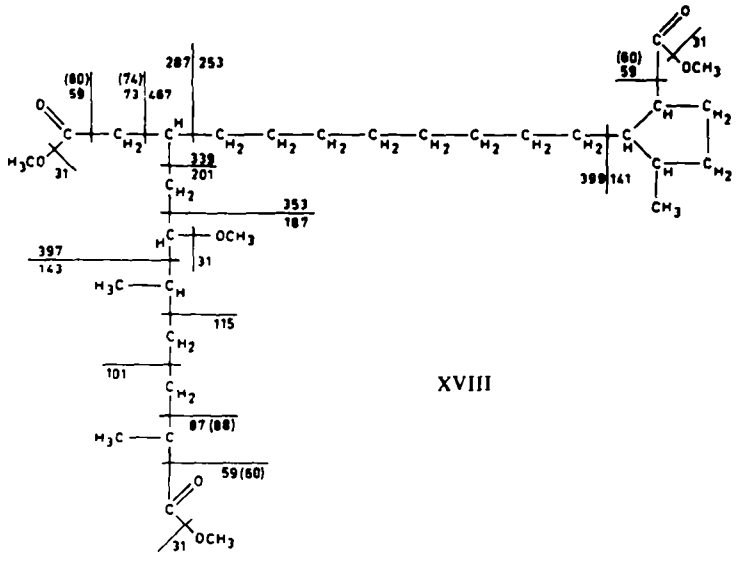
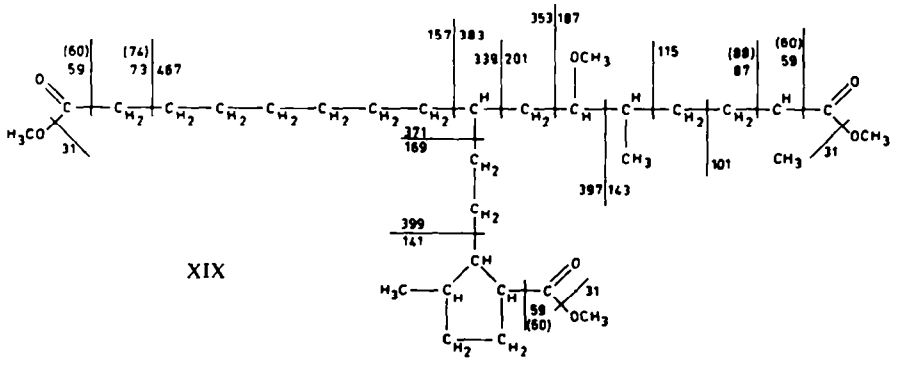


FIG 8. Mass spectrum of HBAMe$_3$



XVIII



XIX

FIG 9

The absence of the expected fragments at $m/e = 201$ and $m/e = 339$ in the MS (Fig. 9) can be explained by the fast fragmentation at the β-position of the OMe group.

Formula XIX accounts for the presence of the fragments $C_{10}H_{17}O_2$ ($m/e = 169$) and $C_{21}H_{40}O_5$ ($m/e = 372$) in the MS (Fig. 9).

$m/e = 169$ $C_{10}H_{17}O_2$

$m/e = 109$ $C_{10}H_{17}O_2(169) — C_2H_4O_2(60) \rightarrow C_8H_{13}(109)$

$m/e = 137$ $C_{10}H_{17}O_2(169) — CH_4O(32) \rightarrow C_9H_{13}O(137)$ $M^* = 111{\cdot}1$

$m/e = 141$ $C_{10}H_{17}O_2(169) — C_2H_4(28) \rightarrow C_8H_{13}O_2(141)$ $M^* = 117{\cdot}6$

Formula XVIII does not give a simple solution for the presence of $m/e = 169$ and $m/e = 372$ in the MS.

Formula XIX leads us to expect that the fragments $m/e = 157$ and $m/e = 383$ should be present. However, the MS does not show these peaks in a reasonable intensity, but derived fragments are probably present (Fig. 9).

$m/e = 97$ $C_9H_{17}O_2(157) — C_2H_4O_2(60) \rightarrow C_7H_{13}(97)$

$m/e = 84$ $C_9H_{17}O_2(157) — C_3H_5O_2(73) \rightarrow C_6H_{12}(84)$

$m/e = 83$ $C_9H_{17}O_2(157)$—$C_3H_6O_3(74) \rightarrow C_6H_{11}(83)$
$m/e = 195$ $C_{22}H_{39}O_5(383)$—$C_{10}H_{20}O_3(188) \rightarrow C_{12}H_{19}O_2(195)$
$m/e = 323$ $C_{22}H_{39}O_5(383)$—$C_2H_4O_2(60) \rightarrow C_{20}H_{35}O_3(323)$

On the basis of formulae XXVIII (Fig. 9) a cleavage of $HBAMe_3$ into $C_3H_5O_2$ ($m/e = 73$) and $C_{28}H_{51}O_5$ ($m/e = 467$) was expected. Fragment $C_{28}H_{51}O_5$ and its derived fragments (467-32.x) do not occur and other fragments expected, $C_{15}H_{27}O_4$ ($m/e = 287$) and $C_{16}H_{29}O_3$ ($m/e = 253$) are not present. Formulae XXVIII and XIX both account for the presence of $C_3H_5O_2$ ($m/e = 73$).

The above data leads us to suggest formula XIX for trimethy ester of HBA and formula XXV for bonkrekic acid.

In Fig. 10 part of the fragmentation pattern for $HBAMe_3$ is shown.

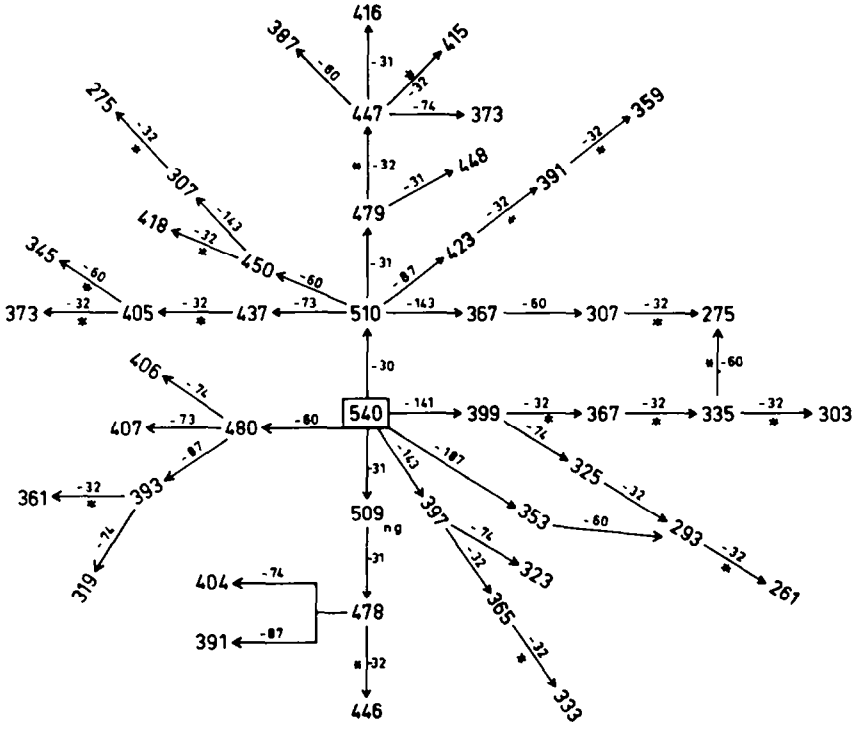
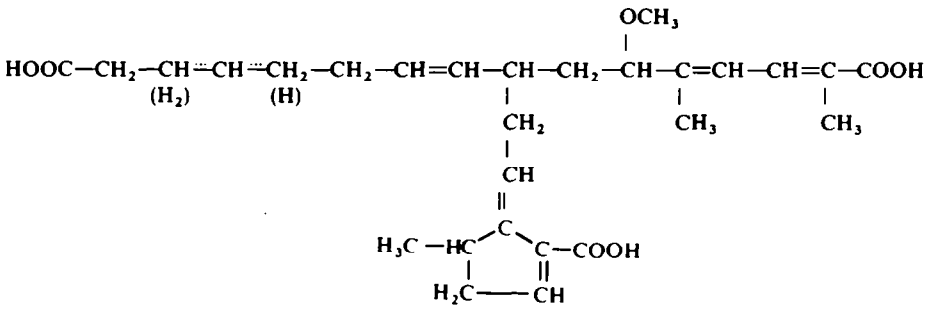


FIG 10

CONCLUSIONS

From chromic acid oxidation experiments three possible formulae for HBA were postulated (XVIII, XIX, XX). Only in two of these formulae six double bonds (of BA) could be placed in such a way that the products formed at ozonolysis and the UV and NMR could be explained (XXIV, XXV). From the MS of $HBAMe_3$ formula XVIII for HBA was rejected. Thus we suggest for bongkrekic acid formula XXV, in

which for one of the isolated double bonds the two possible positions are shown by dotted lines†



XXV. Bongkrekic acid.

EXPERIMENTAL

The UV spectra were recorded with a Bausch and Lomb Spectronic 505, and a Zeiss PMQ 11 spectrometer. The IR spectra (liquid films) were measured on a Unicam SP-200 and Helger-Infrascan. The NMR spectra were recorded on a Varian A-60 and Varian H-220, at room temperature in $CDCl_3$ (TMS). The mass spectra were taken on an AEI-902 mass spectrometer (170° and 70 eV) using the direct insertion probe. For GLC/MS work an AEI MS 12 was used. Fatty acids were separated on a 2 m. column of Porapak Q at a temperature of 190°C. The methylesters of the oxidation products were separated on a 4 m. column of 10% silicon on Chromosorb P 60/80; AW/DMCS at a temperature of 230°C.

Chromic acid oxidation of hydrobongkrekic acid. In a typical oxidation experiment 34 ml of the oxidation mixture (18 g of H_2SO_4, 22 ml of H_2O and 13·2 g of $Na_2Cr_2O_7$) was added to HBA 585 mg. After stirring (room temperature) for 24 hr the reaction was stopped by addition of MeOH. The mixture was extracted continuously (ether) during 72 hr, the etheral solution evaporated, dried, and the residue (443 mg) was esterified with MeOH—5% HCl—10% $(CH_3)_2CO_3$. Yield (425 mg), distilled 90° 10^{-3} mm. As the methylesters of monocarboxylic acids could be lost in the experiments described HBA (575 mg) was oxidized in a separate experiment. The ethereal solution, obtained from continuous extraction of the oxidation mixture, was steam-distilled. The distillate (600 ml) was extracted with n-hexane (100 ml). The n-hexane extract was dried over Na_2SO_4 and evaporated.

Demethoxylation of hydrobongkrekic acid. A mixture of anhydride (10 ml) and p-TsOH (31 mg) was added to $HBAMe_3$ (958 mg) (trimethyl ester was used to prevent lactonization). Mixture heated for two hr (150°), cooled to room temperature, and evaporated. The residue was dissolved in ether (20 ml), extracted with 4% $NaHCO_3$, and washed with water until neutral. The ethereal solution showed two spots on a TLC (silica GF_{254}) R_f of 0·5 and 0·7 ($R_{f\,HBAME_3}$ = 0·6). A mixture of cyclohexane ether (1:1) as solvent, identification with I_2-vapour. The ether was removed and the residue solved in MeOH (25 ml). After ozonolysis (10°) the MeOH was removed. A mixture of formic acid (25 ml) and H_2O_2 (30%, 10 ml) was added to the residue. After the mixture has stood overnight, the excess of performic acid was destroyed by refluxing (30 min). The solution was evaporated to dryness. The residue was esterified by refluxing with dimethyl carbonate (20 ml) and CH_3OH—5% HCl (25 ml) (four hr).

The solution was evaporated and the residue dried. The oil was distilled 90°/10^{-3} mm.

The distillate (140 mg) was investigated with GLC on a 4 m column of 10% silicon on Chromosorb P (190°). The residue (548 mg) was cleaved with chromic acid as for HBA, giving 200 mg of volatile esters, (investigated by GLC).

† Preliminary investigations on the reductive ozonolysis of BA indicate the position of the isolated double bond should be 3–4.

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