ISOLATION OF CUCURBITACIN C FROM *CUCUMIS PROPHETARUM* LINN.

M. GOPALA RAO AND L. RAMACHANDRA ROW
Department of Chemistry, Andhra University, Waltair

ENSLIN *et al.*[1] recorded that cucurbitacins B and D were the main bitter principles of the fruit of *Cucumis prophetarum* Linn. of the South African variety. During our study of the bitter principles of Cucurbitaceæ, the fruits of *C. prophetarum*, collected from the hills around Waltair, were examined. The juice of these fruits was expressed and allowed to settle for some time when a gelatinous matter separated. It was filtered, extracted with alcohol and the extract concentrated, when a colourless crystalline compound was obtained. The pulp of the fruit also gave the same compound when extracted with alcohol. (Yield: 0·03% of the weight of the fresh fruits). Paper chromatography[2] using Whatmann No. 1 filter-paper, previously impregnated with alcoholic formamide and ethyl acetate-benzene (1 : 1) as solvent system showed one spot when sprayed with a 0·5% solution of potassium permanganate in a saturated aqueous solution of cupric acetate. The compound crystallised from ethanol as colourless needles, m.p. 205–07°, $[\alpha]_D^{30} + 72{\cdot}5°$ (*c*, 0·9 ethanol); $\lambda_{max.}$ 230 mμ (ϵ 13500); $\nu_{max.}^{Nujol}$: 3675, 3575, 3485, 3370 (hydroxyl), 1728, 1250 (acetoxyl) and 1692, 1630 cm.$^{-1}$ (α, β-unsaturated ketone). It exhibited the following colour tests: Liebermann-Burchard—pink colour changing to red, tetranitromethane—pale yellow, tetrazolium blue—negative and $FeCl_3$—negative. It analysed for $C_{32}H_{48}O_8$, 1 H_2O. A point of significance is the tenacious retention of a molecule of water by the compound, which could not be removed even at 140°/0·2 mm. It formed an amorphous triacetate, $C_{38}H_{54}O_{11}$, which still contained a free hydroxyl group ($\nu_{max.}^{Nujol}$: 3455 cm.$^{-1}$) and did not form the 2 : 4-dinitrophenylhydrazone.

Hydrogenation over $Pd/CaCO_3$ in ethanol furnished the dihydro derivative, colourless needles from methanol, m.p. 222–24°, $[\alpha]_D^{30}$ + 90° (*c*, 0·8 ethanol), which has no α, β-unsaturated ketone ($\lambda_{max.}$ 289 mμ; $\nu_{max.}^{CHCl_3}$: 1698 cm.$^{-1}$), but still contains a double bond (tetranitromethane—pale yellow), that resisted hydrogenation. The bitter principle and its dihydro derivative consumed one mole of perbenzoic acid, each forming a monoepoxide. The epoxide of the bitter principle, m.p. 180–82°, contains the α, β-unsaturated ketone ($\nu_{max.}^{Nujol}$: 1695, 1641 cm.$^{-1}$). Thus the bitter principle contains two double bonds one of which is unaffected during hydrogenation, but reactive towards perbenzoic acid and the other is conjugated with a ketone; it can be reduced but is unreactive to perbenzoic acid.

Ozonolysis of the bitter principle furnished two aldehydes, 2-hydroxy isobutyraldehyde (II) and 2-methyl acrolein (III), isolated as their 2 : 4-dinitrophenyl hydrazones as in the case of cucurbitacins A, C and E.[3–5] This indicates that it has the same side chain as these cucurbitacins. Oxidation with periodic acid furnished a crystalline methyl ketone, $C_{24}H_{36}O_5$, m.p. 230–32°, $[\alpha]_D^{30} + 180°$ (*c*, 0·98 ethanol) and a water-soluble acid, $C_6H_{10}O_3$ which was obtained as a pale yellow gum that resisted crystallisation. The infra-red spectrum of this acid showed bands at 3500, 1715, 1665 and 980 cm.$^{-1}$ indicating the absence of acetoxyl group which might have hydrolysed during the reaction or subsequent working up. During similar oxidation of cucurbitacins D, E and I, *trans*-4-hydroxy, 4-methyl, pent-2-enoic acid (IV) was previously isolated as a crystalline solid, m.p. 102–103°.[5] In spite of several attempts to purify this gum through chromatography over silica gel, only a pale yellow gum could be secured. Notwithstanding this difference, the acid from the bitter principle bears close resemblance to *trans*-4-hydroxy, 4-methyl, pent-2-enoic acid (IV) and consequently the bitter principle may be considered to possess the same side chain as cucurbitacins D, E and I with an acetoxy function at C-25.

The methyl ketone (V), $C_{24}H_{36}O_5$ formed a triacetate, (pyridine-acetic anhydride), needles from ethanol, m.p. 158–60°, $[\alpha]_D^{30} + 118°$ and when treated with protonic agents like *p*-toluene sulphonic acid or methanolic hydrochloric acid underwent dehydration giving anhydrohexanor compound (VI, R=CH_2OH), m.p. 179–80°, ($\lambda_{max.}$ 242 mμ; $\nu_{max.}^{CHCl_3}$ 1660, 1590 cm.$^{-1}$). When treated with 1 N alcoholic alkali for 18 hr. at room temperature, both the bitter principle and its hexanor compound lost

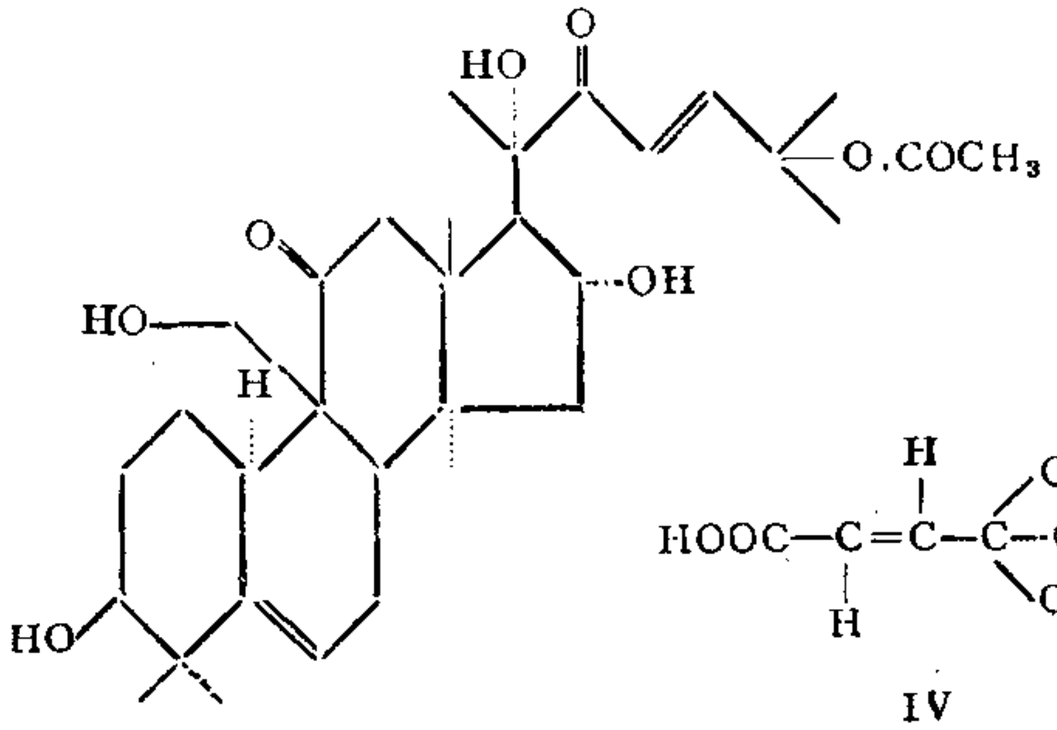
formaldehyde which was identified by chromotropic acid colour test. The physical constants of the bitter principle and its reaction products agree well with those of cucurbitaein C (I) and its reaction products (Table I).

(c, 0·5 ethanol) and spectral data ($\lambda_{max.}$ 242 mμ ; $\nu_{max}^{CHCl_3}$ 1740, 1710 and 1668 cm.$^{-1}$) agree well with those of cucurbitone C (VII). The anhydrohexanor compound (VI, R=CH_2OH), when treated with alcoholic alkali under

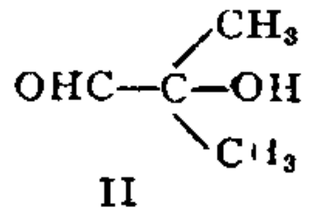
Table I

Reaction	Product	Bitter principle	Cucurbitacin C
1.		m.p. 205-07° $[\alpha]_D$ +72·5°	207–207·5° +95°
2. Catalytic reduction ..	Dihydro derivative	m.p. 222–24° $[\alpha]_D$ +90°	226° +66°
3. HIO_4 oxidation ..	Hexanor compound	m.p. 230–32° $[\alpha]_D$ +180°	235–36° +185°
	-acetate	m.p. 158 60°	157–58°
4. Action of CH_3OH—HCl on hexanor compound	Anhydrohexanor compound	m.p. 179–80°	178–79°

In order to confirm the identity, two more reactions were performed. The bitter principle triacetate on oxidation with chromium trioxide-acetic acid gave a crystalline product, whose physical constants, m.p. 232–35°, $[\alpha]_D$ + 160° reflux decomposed to give formaldehyde (detected by chromotropic acid test and dimedone derivative) and Δ^{16}-anhydroheptanor cucurbitacin C (VI, R = H), $C_{23}H_{32}O_3$, m.p. 248–52°, $[\alpha]_D^{30}$ — 180° (c, 0·8 ethanol).

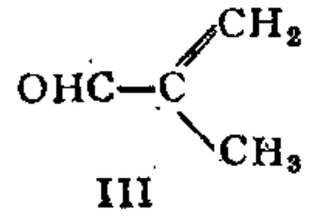


I

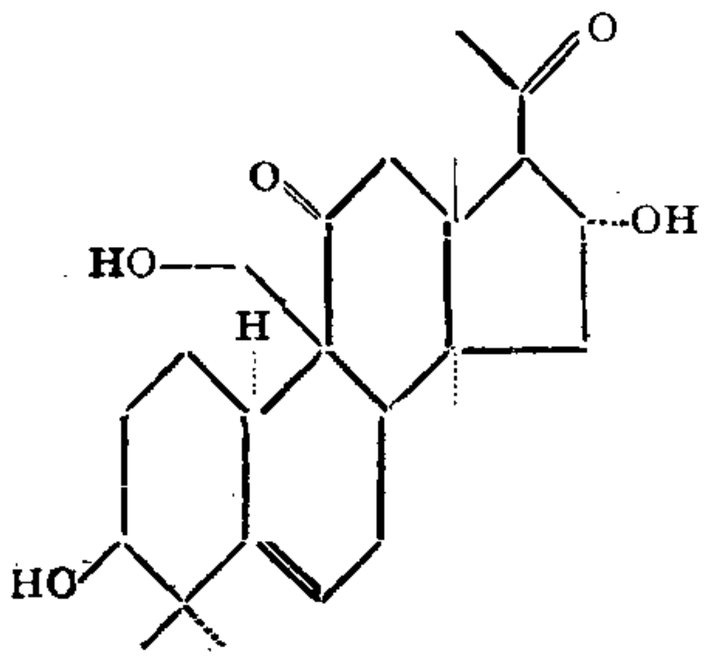


II

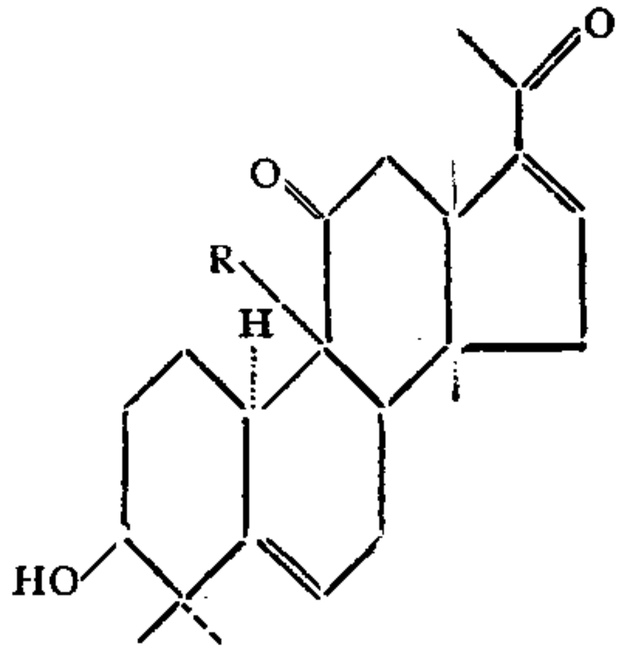
IV



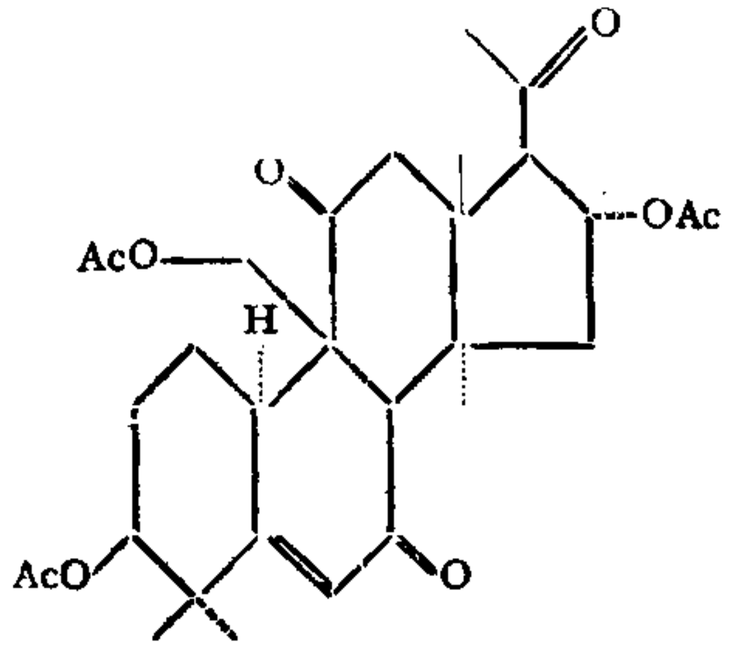
III



V



VI



VII

The N.M.R. spectra of the bitter principle and some of its reaction products were studied and they fully support the identification of the bitter principle as cucurbitacin C (I). Some of the important signals of the N.M.R. spectra are presented in Table II.

TABLE II

N.M.R. spectral data of the bitter principle and its reaction products

Solvent : $CDCl_3$, Internal standard : Tetramethylsilane

Compound	Chemical shift τ scale		Groups assigned
1. Bitter principle* (Cucurbitacin C) ..	3·1	s	Olefinic H at C-23 and C-24
	4·30	m	Olefinic H at C-6
	7·98	s	Acetate methyl
2. Dihydrocucurbitacin C* ..	4·36	m	Olefinic H at C-6
	7·98	s	Acetate methyl
3. Hexanor cucurbitacin C triacetate ..	4·25	m	Olefinic H at C-6
	5·30, 6·05	AB q J = 11 cps	$-CH_2-OAc$
	7·82, 7·93, 7·96, 8·00	s	Methyl ketone and three acetate methyls
4. Δ^{16}-Anhydrohexanor cucurbitacin C diacetate	3·28	t, J = 2 c/s	Olefinic H at C-16
	4·25	m	Olefinic H at C-6
	5·35, 5·99	AB q J = 11 cps	$-CH_2.OAc$
	7·92, 8·17, 8·21	s	Methyl ketone and two acetate methyls
5. Cucurbitone C ..	3·78	d J = 2 cps	Olefinic H at C-6
	4·37	t, J = 7·5 cps	$-CH-O.CO.CH_3$ C-16
	5·32	m	$-CH-O.CO.CH_3$ C-3
	5·53, 6·04	AB q, J = 11·5 cps	$-CH_2.OAc$
	7·86, 7·95, 8·04, 8·10	s	Methyl ketone and three acetate methyl
6. Δ^{16}-Anhydroheptanor cucurbitacin C	3·18	t, J = 2·5 cps	Olefinic H at C-16
	4·42	m	Olefinic H at C-6
	7·68	s	Methyl ketone

* Spectra taken in deuterated DMSO, s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet.

Finally our identification was confirmed by direct comparison (m.m.p., I.R., and paper chromatography) of the bitter principle and its hexanor compound with authentic cucurbitacin C and hexanor cucurbitacin C, kindly made by Dr. P. R. Enslin. It may be mentioned that cucurbitacin C has so far been reported to occur in *Cucumis sativus* only.[7] The present isolation from *C. prophetarum* is the second instance of its occurrence.

ACKNOWLEDGEMENTS

We are highly indebted to Dr. P. R. Enslin, National Chemical Research Laboratory, Pretoria, South Africa, for kindly comparing our samples with cucurbitacin C and hexanor cucurbitacin C, to Prof. A. J. Birch and Dr. G. S. R. Subba Rao, University of Manchester, for the N.M.R. spectra and to the University Grants Commission, for the award of Junior Research Fellowship to one of us (M. G. Rao).

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