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THE STRUCTURE OF PHRAGMALIN A MELIACIN WITH A NORBORNANE PART SKELETON

R. R. ARNDT* and W. H. BAARSCHERS†

National Chemical Research Laboratory, C.S.I.R., Pretoria, Republic of South Africa

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Abstract—Structure II for phragmalin, a meliacin isolated from *Entandrophragma caudatum* Sprague is proposed on the basis of chemical and spectroscopic evidence.

TWO BASIC CONSTITUENTS were isolated from the bark of *Entandrophragma caudatum* Sprague by the conventional alkaloid extraction method.

On alkaline hydrolysis both these basic compounds gave rise to the same three acids, *viz.* iso-butyric, nicotinic and a third acid with a molecular formula $C_{28}H_{34}O_{11}$. The methyl ester of the latter analysed for $C_{29}H_{36}O_{11}$ and was named phragmalin (II). The origin[1] and molecular complexity suggested that phragmalin belongs to the meliacins also known as tetranortriterpenes.

The presence of the commonly occurring β-substituted furan ring in meliacins was evident from spectroscopic data. In the PMR spectrum‡ the signals at δ 7·51, 7·35 and 6·42 are typical of a β-substituted furan moiety.[2] In the UV spectrum the maximum at 209 nm (ε 5674) is assigned to a furan chromophore.[3] The mass spectrum of phragmalin shows *inter alia* prominent peaks at *m/e* 464 ($C_{24}H_{32}O_9$), *m/e* 121 (C_8H_9O) and *m/e* 95 ($C_5H_3O_2$) represented by *a*, *b* and *c*, respectively, in Scheme 1. These fragments are indicative of the presence of a β-substituted furan group in the molecule.[4, 5]

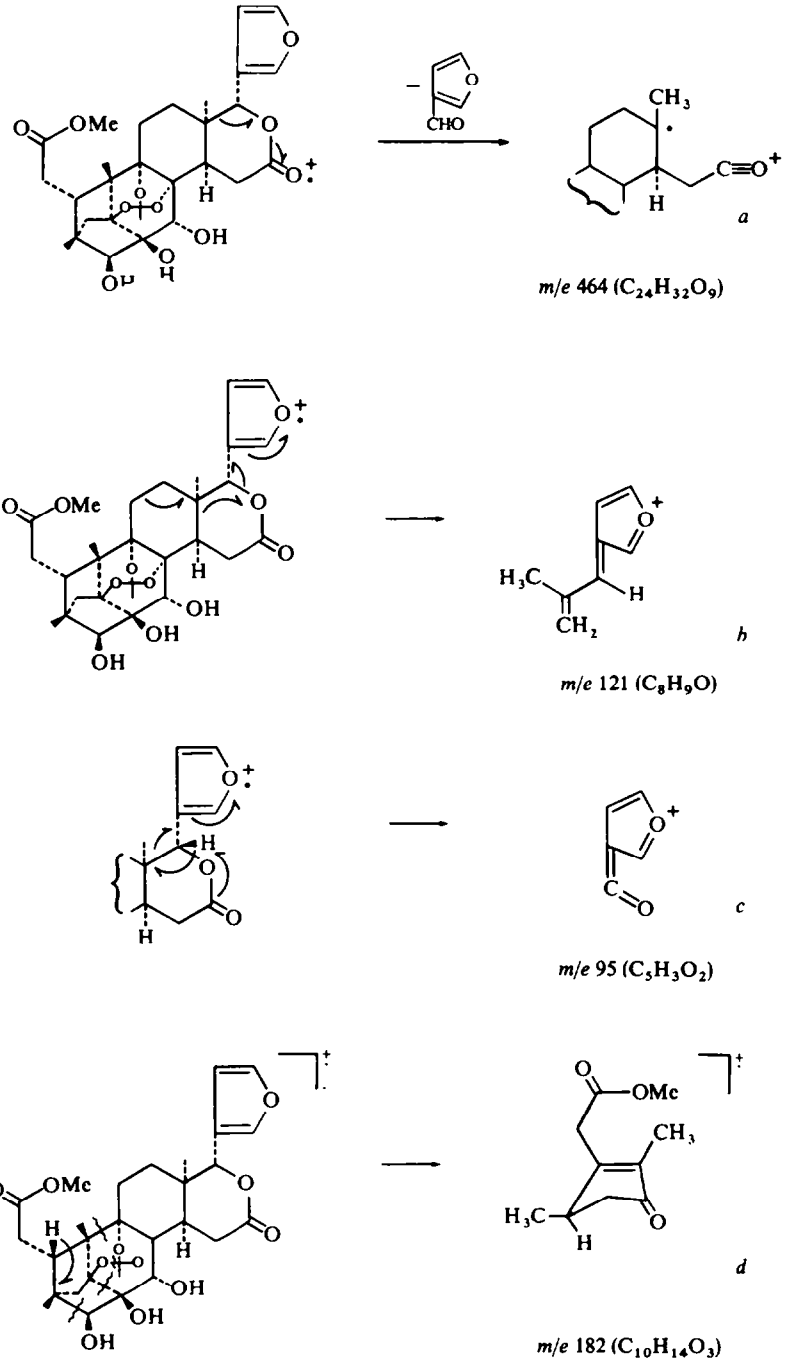
Further support for the presence of a β-substituted furan group in phragmalin came from the spectroscopic data of tetrahydrophragmalin, the product of catalytic hydrogenation of phragmalin (Experimental).

The IR spectrum showed two carbonyl absorption bands at ν 1723 cm^{-1} and 1743 cm^{-1} ascribed to the presence of a carbomethoxy-group (PMR δ 3·64) and a δ-lactone group, respectively. The singlet in the PMR at δ 5·53 is characteristic[2] of H-17 in meliacins. The PMR spectrum of phragmalin also revealed an AMX pattern of signals consisting of two triplets centred at δ 3·55 (A) and δ 1·88 (X) and a quartet at δ 2·60 (M). H_M shows two large coupling constants J_{AM} and J_{MX} 18·5 Hz and 10·0 Hz, respectively. The larger coupling is assigned to a geminal coupling of protons adjacent to a ketone. The lower signal (A) is that of a proton lying in the plane of the carbonyl bond.[6] This AMX system was accommodated by the δ-lactone ring in part structure (I).

* Present address: Department of Chemistry, Rand Afrikaans University, Johannesburg, Republic of South Africa.

† Present address: Department of Chemistry, Lakehead University, Thunder Bay, Ontario, Canada.

‡ PMR data refer to deuteriochloroform solutions except where otherwise stated and are in ppm from TMS as internal standard run on a Varian HR-100 mH instrument.


OMe
OH
OH
OH
CHO
CH3
C≡O+
a
m/e 464 (C24H32O9)
H3C
CH2
b
m/e 121 (C8H9O)
C=O
c
m/e 95 (C5H3O2)
d
m/e 182 (C10H14O3)

SCHEME 1

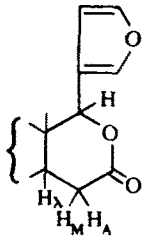
Evidence for the presence of three OH groups in phragmalin came from active hydrogen analysis, mass spectroscopy and PMR data. The PMR spectrum of phragmalin also showed that two of the OH groups are on secondary carbons and one OH group is on a tertiary carbon. The PMR spectrum also revealed that the carbon atoms bearing the secondary alcohol groups are flanked by quaternary carbons. Mild acetylation (pyr./Ac_2O, 25°) yielded a monoacetate $C_{31}H_{38}O_{12}$ (III), while under more drastic conditions (Ac_2O/TsOH) a triacetate $C_{35}H_{42}O_{14}$ (IV) was obtained. The IR spectrum of the latter was free of the OH absorption band, in the region ν 3400–3560 cm^{-1}, present in the spectrum of phragmalin. Phragmalin consumed two moles of HIO_4 whereas the monoacetate consumed one mole of this reagent. Therefore it can be concluded that the three OH groups are on three adjacent carbon atoms with the tertiary alcohol on the central one.

Chromic acid oxidizes phragmalin to the monoketone phragmalone $C_{29}H_{34}O_{11}$ (V). The monoacetate, however, was stable to chromic acid treatment. The relative inertness of the second secondary alcohol group was ascribed to its participation in a strong hydrogen bond. This conclusion was supported by the observation in the PMR spectrum where doublets appeared at δ 8·38 and δ 4·7 (J 7 Hz), these signals being due to a hydroxyl proton and the hydrogen on the carbon bearing this hydroxyl group, respectively.

The three oxygens not yet accounted for are chemically unreactive and are therefore assumed to be ether oxygens. That these three oxygens are involved in an ortho-acetate grouping was evident from the Me singlet at δ 1·58,[7,8] the loss of 60 mass units ($C_2H_4O_2$) in the MS of phragmalin and the fact that phragmalin possesses two carbon atoms more than normally found in meliacins. The PMR spectrum clearly indicated that the ether oxygens must be attached to tertiary carbon atoms.

Carboxylic acid groups in the meliacin series generally result either from the oxidation of one of the *gem*-dimethyl groups on C-4, *e.g.* hirtin[9] or from biogenetic cleavage of ring B, *e.g.* swietenine.[10] The acid obtained either from the hydrolysis of the isolated base or from phragmalin has a *pKa* value of 6·35 (50% aq acetone), a value that excluded the presence of an oxygen on the carbon α to the carboxyl group. A study of the MS of phragmalin and its deuterated derivatives gave valuable information regarding the environment of the carbomethoxy-group. By the mass shift technique it was shown that the prominent peak at *m/e* 182 ($C_{10}H_{14}O_3$), *d* scheme 1, in the MS of phragmalin contains the methyl ester group. In the spectrum of demethyl-phragmalin the corresponding peak appeared at *m/e* 168 and in the spectrum of the CD_3-ester prepared from deuterated diazomethane[12] it appeared at *m/e* 185. Under enolizing conditions phragmalin exchanged four hydrogens for deuterium: two at C-15 (*m/e* 464 → 466) and the remaining two at C-6, the carbon next to the ester carbonyl (*m/e* 182 → 184). The acid group in phragmalin is therefore analogous to that of swietenine. The PMR spectrum of phragmalin showed besides the ortho-acetate methyl, only three quaternary attached C-Me groups (δ 0·90, 1·01, 1·10), in contrast to the four Me groups present in swietenine. According to the molecular formula and the above data phragmalin must be tetracarbocyclic. This leads to the conclusion that one of the carbons normally present as a Me group in the meliacins must be part of a carbon ring in phragmalin to give rise to a novel structure in the swietenine series.

Final proof of the structure of phragmalin was obtained from an X-ray crystallo-



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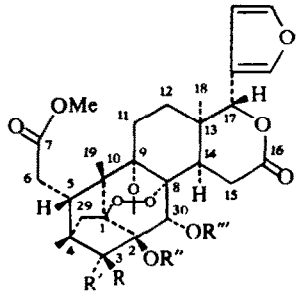
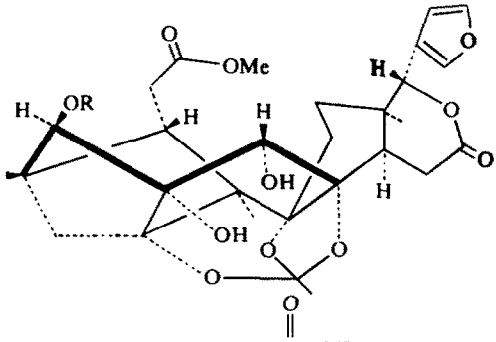


II R = OH, R′ = H, R″ = R‴ = H
III R = OAc, R′ = H, R″ = R‴ = H
IV R = OAc, R′ = H, R″ = R‴ = Ac
V R, R′ = O, R″ = R‴ = H
VI R = —O—C—CH_2I, R′ = H, R″ = R‴ = H
(O)

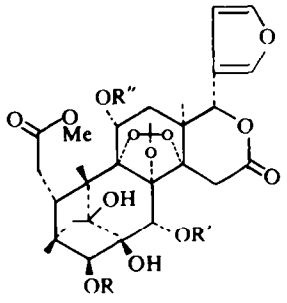
graphic analysis of the iodoacetate of phragmalin (VI).[13] This study revealed the molecular structure of phragmalin as II.

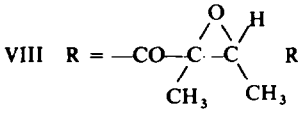
In this structure the C-29 forms a carbon bridge between C-4 and C-1. The most striking stereochemical features of the structure for phragmalin iodoacetate (VII) is the norbornane moiety and the presence of an orthoacetate grouping. These features considered together with a non-bonded interaction between the C-18 Me group and the orthoacetate grouping are responsible for adoption of boat conformations by three contiguous rings (*cf.* VII).

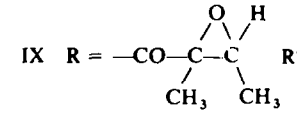


VII R = —C—CH_2I
(O)

Extraction of the heartwood of *Entandrophragma caudatum* yielded entandrophragmin, a meliacin also isolated from other *Entandrophragma* species.[1] That a close structural relationship exists between phragmalin and entandrophragmin was evident from the PMR and MS data; in particular the occurrence of the prominent peaks at *m/e* 182 ($C_{10}H_{14}O_3$) and *m/e* 121 (C_8H_9O) in the mass spectrum, the C-Me







signal at δ 1·82 (orthoacetate) and the signals for only three nuclear Me groups in the PMR spectrum of entandrophragmin. Therefore it was not surprising that the revised structure for entandrophragmin (VIII) recently proposed on the basis of X-ray crystallographic analysis of utilin[14] (IX) is very similar to that of phragmalin. The elucidation of the structures of phragmalin and entandrophragmin adds another novel skeleton to the meliacins, a family of compounds already rich in complex structures.

EXPERIMENTAL

UV absorption refers to EtOH and IR absorption to $CHCl_3$ solns. UV spectra were recorded on a Unicam Model S.P. 800 spectrometer and IR spectra on a Perkin-Elmer Model 237 spectrometer. Mass spectra were taken on an A.E.I. MS-9 spectrometer. The PMR spectra were recorded on a Varian HR-100 mH instrument. Ehrlich spray reagent was used for the development of spots on all TLC plates (0·25 mm silica gel).

Isolation of bases. Finely ground dry bark of *Entandrophragma caudatum* Sprague (240 kg) was extracted with hot EtOH for 24 hr. The EtOH was removed *in vacuo* and the concentrate diluted with twice its volume of water and extracted exhaustively with ether. This ether extract was washed with water and extracted with H_2SO_4 (0·5 N). The acid extracts were basified with NH_4OH and extracted with CH_2Cl_2 to yield the crude basic material (60 g). A similar extraction on the leaves (200 kg) gave the same mixture of bases (20 g) and thus the two extracts were combined.

Separation of mixture of bases. The crude mixture of bases (13 g) was chromatographed on a formamide-impregnated cellulose column (500 g). Elution with hexane–EtOAc (12:5) resulted in partial separation of two basic components, A (1·46 g) and B (0·90 g), and a mixture of the two (2·36 g). Elution with $CHCl_3$–hexane–EtOH mixture of increasing EtOH content afforded more polar material (4·4 g) which was not further investigated. Rechromatography of the A/B mixture led to further separation so that final yields were 15% A and 7% B, calculated on total crude mixture. Base A with picric acid in MeOH gave a crystalline picrate, m.p 125–129° (decomp) from MeOH. (Found: C, 55·7; H, 5·2; N, 6·5. $C_{45}H_{48}N_4O_{20}$ requires: C, 56·0; H, 5·0; N, 5·8%).

The free base liberated from picrate had $[\alpha]_D$ −98° (*c*, 1·8, MeOH), λ_{max} 264 and 217 nm (ε 3334 and 17,280, respectively). (Found: C, 63·8; H, 6·3; N, 1·8. $C_{39}H_{45}NO_{13}$ requires: C, 63·7; H, 6·2; N, 1·9%).

Hydrolysis of base A, determination and identification of volatile acids. The base (728 mg) in EtOH (15 ml) and aqueous KOH (0·3 N, 15 ml) was refluxed for 15 min, the EtOH removed *in vacuo* and the aqueous residue extracted with CH_2Cl_2 to remove basic material. The aqueous layer was acidified and extracted with CH_2Cl_2. The CH_2Cl_2 solution was extracted with KOH (2 N) and the combined extracts made up to 50 ml. Aliquots of this solution were acidified and distilled. The distillate (collected in standard alkali and back-titrated with acid) contained 1 mole of volatile acid. (Found: 0·89, 0·91, 1·02 and 0·96 mole).

The combined distillates after acidification were extracted with CH_2Cl_2. After solvent evaporation the residue was treated with *p*-phenyl-phenacyl bromide.[16] The crystalline product obtained from MeOH was identical with *p*-phenyl-phenacyl-iso-butyrate (m.p., IR, VPC, 6 ft 20% SE 30 on Chromosorb W, at 220°).

Isolation and identification of non-volatile acids. The aqueous acidified hydrolisate on standing deposited crystals (200 mg) which on recrystallization from wet $CHCl_3$ had m.p. 180–185° (decomp). [Found: C, 59·6; H, 6·5; M^+, 546·210 $C_{28}H_{34}O_{11}\cdot H_2O$ requires: C, 59·6; H, 6·4%; M, 546·210]. Titration with NaOH (0·005 N) gave *pKa* 6·35 (acetone-water 1:1). Treatment with ethereal CH_2N_2 afforded *phragmalin* which crystallized from wet ether and had m.p. 150–155° (decomp). $[\alpha]_D$ − 77 (*c*, 1·3, MeOH). λ_{max} 263 and 209 nm (ε 59 and 5673, respectively). Sap Equiv. of 1·9 and 2·0 mole found on hydrolysis. (Found: C, 62·1; H, 6·3. $C_{29}H_{36}O_{11}$ requires: C, 62·2; H, 6·5%).

Zeisel analysis showed that phragmalin contained one OMe group. Phragmalin gave an orange Ehrlich colour reaction.

The aqueous mother liquor of demethylphragmalin was continuously extracted with ether (48 hr). The ether after drying and evaporation yielded a residue (300 mg) which was treated with CH_2N_2. The product consisted of two components (TLC), one being Dragendorff reagent positive, the other giving an orange Ehrlich reagent colour reaction. A base-neutral fractionation afforded methyl nicotinate (30 mg), m.p. 36°, M^+ 137, identical to an authentic sample, and phragmalin.

Trans esterification of base A. To the base A (75 mg) in cooled abs. MeOH (20 ml), a small piece of Na-metal was added, and the solution kept at 0° for 48 hr. The solution was neutralized and solvent removed *in vacuo* at room temp. Addition of H_2O and extraction with CH_2Cl_2 afforded a product from which methyl nicotinate was removed by sublimation *in vacuo*. The residue (52 mg) was recrystallized from wet ether and had m.p. 150–155°, identified as phragmalin.

Hydrolysis of base B. Base B on hydrolysis gave the same three acids, *viz.* iso-butyric, nicotinic and demethylphragmalin.

Catalytic hydrogenation of phragmalin. Phragmalin (150 mg) in AcOH (20 ml) was hydrogenated at room temp and atmospheric pressure in the presence of pre-reduced PtO_2 catalyst. Hydrogen, equivalent to 2·6 moles, was absorbed over a 20 hr period. Recovered material (145 mg) was a mixture of neutral *tetrahydro-* and *hexahydrophragmalin* (M^+ 564 and 566). Separation on silica (10 g) with 2% MeOH in $CHCl_3$ gave pure tetrahydrophragmalin (63 mg), m.p. 132–140° from ether, λ_{max} 214 nm (ε 149), $[\alpha]_D$ − 71° (*c*, 0·7, EtOH). (Found: C, 59·5; H, 7·3. $C_{29}H_{40}O_{11}\cdot H_2O$ requires: C, 59·8; H, 7·3%).

Further elution gave hexahydrophragmalin (4 active hydrogens) contaminated with some tetrahydro-derivative.

Phragmalin monoacetate (III). Phragmalin (100 mg) in Ac_2O (2 ml) and pyridine (2 ml) was kept at room temp for 20 hr. The addition of crushed ice, removal of pyridine *in vacuo* and $CHCl_3$ extraction yielded a foam which was chromatographed on silica. Elution with CH_2Cl_2—C_6H_6 (3:2) gave *phragmalin monoacetate* (60 mg) m.p. 247–253° (decomp) from acetone, $[\alpha]_D$ −78° (*c*, 2·7, EtOH). (Found: C, 61·8; H, 6·4. $C_{31}H_{38}O_{12}$ requires: C, 61·8; H, 6·4%).

Phragmalin di- and triacetate. To phragmalin (25 mg) in AcOH (2 ml) was added Ac_2O (0·5 ml) and *p*-TsOH

(50 mg). After standing for 12 hr at room temp H_2O was added and the mixture extracted with $CHCl_3$. The extract, after being washed with $NaHCO_3$ aq, gave a product (20 mg) which was separated on silica chromatoplates. The two major components were *phragmalin diacetate* (8·5 mg), m.p. 151–158° from acetone, ν_{max} 3575 cm^{-1}. [Found: M^+ 644·249. $C_{33}H_{40}O_{13}$ requires: M, 644·247] and *phragmalin triacetate* (IV) (4·0 mg), m.p. 212–215° from acetone, no ν_{max} between 3700–3400 cm^{-1}. (Found: M^+ 686·260. $C_{35}H_{42}O_{14}$ requires: M, 686·257).

Exchange of hydrogen atoms α to enolizable carbonyl functions of phragmalin with deuterium. Phragmalin (9 mg) was dissolved in CH_3OD prepared according to Streitwieser,[17] and a small piece of Na was added with cooling. The mixture was kept at 0° for 12 hr, the CH_3OD removed *in vacuo*, D_2O (1 ml) added, and the mixture neutralized by adding DCl dropwise. The mixture was extracted with CH_2Cl_2 to give partially deuterated phragmalin (7·5 mg). This process was repeated twice and the final product (5 mg), when filtered through a short column of silica, gave a single spot on TLC, identical R_f value with phragmalin. MS indicated that four protons had been partially exchanged (d_0 = 0, d_1 = 8, d_2 = 32, d_3 = 39, d_4 = 21%).

Trideuteriomethylester of demethylphragmalin. D_2O (5 ml) in which Na (1 g) had been dissolved, was added to a suspension of nitrosomethylurea (1 g) in ether (10 ml). The partly deuterated diazomethane formed was distilled into a mixture of dry THF (20 ml) and dry ether (5 ml). To this solution was added D_2O (2 ml) and phenol-*O*-d (1 g). The top layer was separated and again treated with D_2O. The mixture containing the deuterated diazomethane stood for 1 hr at room temp in the dark, then 5 ml was added to a solution of demethylphragmalin (5 mg) in CH_3OD. This solution remained for 1 hr in the dark at room temp after which the organic solvents were removed by distillation. The product (4 mg) gave a single spot with an R_f identical to that of phragmalin. The MS revealed the partial exchange of three protons as follows: d_0 = 0, d_1 = 9, d_2 = 19, d_3 = 72%.

Oxidation of phragmalin with CrO_3 *to phragmalone* (V). Phragmalin (12 mg) in acetone (1 ml) and excess CrO_3 reagent (0·8 N in 8 N H_2SO_4) was kept at 0° for 20 hr. Excess reagent was destroyed with iso-propyl alcohol. The organic solvents were removed *in vacuo*, aqueous Na_2CO_3 added and the mixture extracted with CH_2Cl_2. TLC (3% MeOH in $CHCl_3$) indicated that the product consisted of one major product which was less polar than phragmalin, m.p. 245–250° from MeOH, ν_{max} 3530, 1720–1750 cm^{-1}. (Found: C, 62·1; H, 6·2. $C_{29}H_{34}O_{11}$ requires: C, 62·4; H, 6·1%).

Deuterium exchange on phragmalone. Phragmalone (8 mg) was subjected to deuterium exchange under enolizing conditions as described above for phragmalin. The treatment was repeated twice. The product was purified by TLC. The MS indicated partial exchange of four protons as follows: d_0 = 0, d_1 = 0, d_2 = 4, d_3 = 14, d_4 = 82%.

Oxidation of phragmalin with periodic acid. Phragmalin (10·9 mg; 12·1 mg) was dissolved in EtOH (20 ml) and H_2O (10 ml). To the solution was added HIO_4 aq. (0·5 M; 5 ml) and the mixture made up to 50 ml with distilled water. After being kept for 36 hr at room temp in the dark, 10 ml aliquots were taken, and treated with sodium arsenite aq. (0·02 N; made up in 20% $NaHCO_3$, 7·5 ml) and KI aq. (20%; 1·5 ml) After standing for 10 min the solution was titrated with I_2 solution (0·018 N); starch was used as indicator. A blank experiment was run simultaneously and the titration difference (1·06 ml and 1·19 ml) indicated a consumption of 2·42 and 2·47 moles of periodate, respectively. Following the identical procedure on phragmalin monoacetate a titre difference indicated consumption of 1·1 mole.

Phragmalin iodoacetate (VI). Phragmalin (36 mg) was treated with monochloroacetic anhydride (74 mg) in C_6H_6 (0·5 ml) in the presence of pyridine (0·08 ml) for 48 hr at 20°.

The solution was extracted with CH_2Cl_2 and the product separated on TLC (5% MeOH in CH_2Cl_2) to yield *phragmalin chloroacetate* (23·4 mg), M^+, 636 and 638. The chloroacetate (23·4 mg) was dissolved in acetone, KI (170 mg) was added and the mixture refluxed for 5 hr. The solvent volume was reduced *in vacuo*, the residue distributed between H_2O and CH_2Cl_2. The latter afforded the *iodoacetate of phragmalin* (VI) which was purified by TLC as described above, m.p. 138–140° from CH_2Cl_2–hexane, M^+ 728.

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