ISOLATION OF 1,2-DIHYDROXY-4-GLUCOSYLOXYNAPHTHALENE FROM LAWSONIA INNERMIS

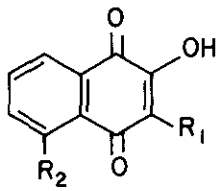
Muhammad Afzal*, Galib Al-Oriquat, Jassim M. Al-Hassan, Nazar Muhammad

Biochemistry Department, Kuwait University, Kuwait

Abstract - Isolation and characterization of 1,2-dihydroxy-4-glucosyloxynaphthalene from L. innermis are described for the first time.

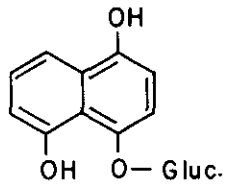
Lawsonia innermis (syn. L. alba Lam., Lythraceae), commonly known as henna, is an ancient plant and is often used as a folklore medicine throughout Asia, the Middle East and other countries. The leaves of this plant are astringent and are used as prophylactic against skin diseases[1]. The leaves were shown to have some action against tuberculosis, typhoid and haemorrhagia[1]. The antibacterial activity of an aqueous extract of henna leaves has been demonstrated by Malekzadeh[2].

The plant leaves are mainly used as cosmetic for staining hands, feet and hair. The dye present in this plant has been characterized as 2-hydroxy-1,4-naphthoquinone (lawsone[3]). The presence of 1,3-dihydroxynaphthalene has also been predicted in this plant[4]. Lawsone (I) and related lapachol (II) are shown to have similar antimicrobial activities[5]. Both are effective against Brucella species and Neisseria catarrhabis, the minimum inhibitory concentration of lawsone ranging 50-200 µg/ml. In vivo, lawsone showed an antitumor activity against SARCOMA-180 in mice[5].



(I) : $R_1 = R_2 = H$

(II) : $R_1 = CH_2 \cdot CH : CMe_2$; $R_2 = H$



(III)

Lawsone was first isolated by Tommasi by alkaline extraction of L. alba, in 1920. Subsequently this compound was isolated from other species such as L. spinosa L; Impatiens balsamina L;

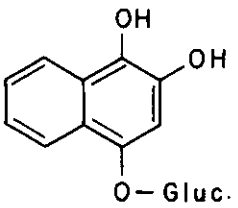
Ipallida nutt and I. capensis[3]. The presence of a reduced form of lawsone has been indicated in I. balsamina L. by Glennie and Bohm[6]. Similarly the presence of 1,5-dihydroxy-4-glucosyloxynaphthalene (III) in green walnuts has been reported by Hayes and Thomson[7]. In view of the increasing evidence of the pharmacological activity of L. alba, we decided to reinvestigate the components of this plant for the possible presence of reduced lawsone which could easily autooxidize to give lawsone.

All previous reports describe the extraction of L. alba using aqueous or alkaline conditions[4]. These extraction procedures are responsible for the autooxidation of the reduced quinone present in this plant. As a test of this we extracted L. alba with purified methanol and other solvents and found that lawsone (I) was not at all present in any one of these extracts. However an alkali treatment of the methanolic extract showed the presence, against an authentic sample, of lawsone (I), on tlc. We thus conclude that lawsone (I), previously described as a metabolite of L. alba is in fact an artifact. Attempts were therefore concentrated to isolate the probable reduced quinone from this plant.

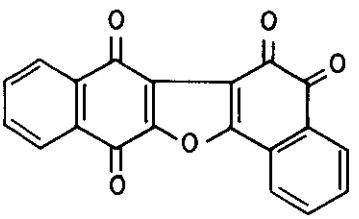
L. alba leaves (1 kg), collected from Ministry of Health, Kuwait, were air dried and powdered. It was then exhaustively extracted by Soxhlet extraction apparatus using pure methanol as a solvent. The solvent was evaporated under reduced pressure to give a dark viscous gum which did not show the presence of lawsone on tlc. However alkaline treatment of the extract showed presence of lawsone.

The concentratred methanolic extract was chromatographed on a series of silica-gel columns by gradient elution. Light petroleum ether-chloroform (8:2 v/v) fractions yielded β-sitosterol mp 138° C, lit.[8] mp 136.5° C and stigmasterol mp 170-172° C, lit.[9] mp, 170-171° C. Subsequent ethylacetate fractions yielded a light brownish gum (110 mg) which could not be crystallized from a variety of organic solvents. However under high vacuum it turned into an amorphous powder which returned to a gum form after being left for some time. It showed a single spot on tlc (EtOAc:EtOH:AcOH, 17:2:1 v/v) and gave a dark brown ferric chloride reaction. It was highly soluble in water and insoluble in most organic solvents. Saponification of the gum under alkaline conditions gave lawsone and glucose in quantitative yields as evidenced by paper chromatography. ir ν_{max}. 3450-3200 (OH); 1635, 1605, 1595 cm^{-1} (C=C, Ar). NMR (acetone-D_6)τ: 1.55q, 1.85q, 2H, (Ar-H, at C_5 & C_8); 3.3s, 1H, (Ar-H, at C_3); 4.95 br, 4H, (Glycosyl-OH); 6.2m, 7H, (Glycosyl-H). The natural product was unstable and changed into a red, insoluble material, after exposure to light and air. However it gave a stable hexaacetate derivative as a colourless amorphous powder, mp 83-85° C decomposing at 95-97° C.

The natural product had an uv absorption spectrum similar to that of 1,2,4-trihydroxynaphthalene[10]. This indicated that this product was a glucoside of 1,2,4-trihydroxynaphthalene. The glycosyl link at C_4 is favoured since glycosyl link at C_2 will give a stable 1,4-naphthoquinone-2-glucoside. The palcement of glycosyl link at C_1 is also not possible since the resulting compound should be resistant to autooxidation. However placement of glycosyl link at C_4 will give a compound compatible with the properties mentioned above. The compound is thus characterized as 1,2-dihydroxy-4-glucosyloxynaphthalene (IV).

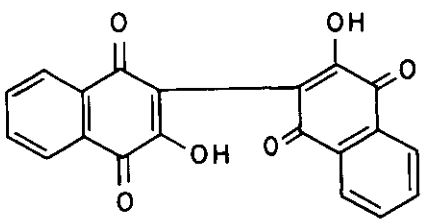


(IV)



(V)

The red insoluble material, obtained after exposure of the natural product (IV) to air and light showed a molecular ion M* 328.0386 ($C_{20}H_8O_5$) requires 328.0382. This indicated the photocatalysed dimerisation of the product (IV) with autooxidation and elimination of the sugar residue. The product, mp 318° C with decomposition, was characterized (MS, ir and mixed mp) as α,β'-anhydride (V), lit.[11] mp 318°C. This anhydride (V) has been previously prepared[11] by ultra violet irradiation of lawsone to give intermediate 3,3'-dihydroxy-2,2'-binaphthoquinone (VI) which undergoes dehydration to give α,β'-anhydride (V).



(VI)

Antimicrobial activity of the product (IV) was checked against a number of test microorganisms and it was found to be active against Bacillus subtillus Cohn emend. Prazmowski, NRRL B-765 and Sacchromyces Pastorianus Hansen, NRRL Y-139. Compound IV proved to active against psoriasis when applied regularly for seven days on five psoriatic volunteers. No side effect was detected during treatment of this limited number of patients.

ACKNOWLEDGEMENT One of the authors (MA) is thankful to Kuwait University for financial grant SB0 10 to support this work.

REFERENCES

1. S.K. Jain, "Medicinal Plants" National Book Trust, India, 1973, 91.
2. F. Malekzadeh, Applied Microbiology, 968, 663.
3. R.H. Thomson, "Naturally Occurring Quinones", Academic Press, 1971, p. 202.
4. A. Latif, Indian J. Agric. Sci., 1959, 29, 147.
5. O. G. de Lima, Jose, S. de Coelho, I.L. de Albuqerque, J.F. de Mellow, D.G. Martins, A.L. Lacerda and M.A. De M. de Souza, (Brazil) Rev. Inst. Antibiot., Univ. Fed. Pernambuco, Recife, 1971, 11(1), 21; Chem. Abs., 1972, 77, 29629n.
6. C.W. Glennie and B.A. Bohm, Canadian J. Biochem., 1965, 43, 293.
7. N.F. Hayes and R.H. Thomson, J. Chem. Soc., 1955, 904.
8. G. Majumdar and S. Thakur, J. Indian Chem. Soc., 1968, 45, 785.
9. R.J. Anderson and R.L. Shriner, J. Am. Chem. Soc., 1926, 48, 2976.
10. C. Daglish, J. Am. Chem. Soc., 1950, 72, 4859.
11. S.C. Hooker, J. Am. Chem. Soc., 1936, 58, 1212.

Received, 22nd December, 1983