SHORT COMMUNICATIONS

FLAVONE C-GLYCOSIDES FROM *MOLLUGO HIRTA*

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THE genus *Mollugo*[1] was until recently included as a member of the betalain family, the Aizoaceae[2]. The occurrence of anthocyanins and the absence of betacyanins in *Mollugo* prompted the placement of this genus in a separate family Molluginaceae. To find further support, we have examined earlier the flavonoids of all the *Mollugo* species available in South India and found that all of them contained flavone C-glycosides[3].

In continuation of the detailed examination resulting in the isolation of new flavone C-glycosides from *M. disticha*[3] and *M. pentaphylla*[4,5] and rare ones from *M. cerviana*[6], we have studied *M. hirta*[7] (Syn. *M. lotoides*; *M. parviflora*) for its glyco flavonoids and our isolation of vicenin-2 and vitexin is reported here.

Fresh aerial parts of *Mollugo hirta* were extracted with hot 90% ethanol and the flavonoids present in the concentrate was purified[3,4] by column as well as paper chromatography to yield two flavone C-glycosides, A and B.

Compound A crystallised from MeOH as light yellow needles, $C_{21}H_{20}O_{10}$, m.p. 250–52° C. It was purple under UV and light yellow under UV/NH_3, had λ_{max}, 271, 302sh, 333nm (MeOH) and diagnostic shifts[8] typical of apigenin. Its R_f and nonhydrolysability with 25% HCl indicated it to be a flavonoid C-glycoside. On hydrolytic fission with HI, it yielded apigenin and on $FeCl_3$ oxidation gave glucose. The MS of the permethylether gave an intense parent peak (m/z; 530, 84%) typical of apigenin 8-C-glucoside[9]. From these data, compound A was identified as vitexin (apigenin 8-C-glucoside) and the identity further confirmed by direct comparison with an authentic sample[10].

Compound B crystallised from aq. MeOH as light yellow needles, $C_{27}H_{30}O_{15}$, m.p. 265–66° C. It was purple under UV and light yellow under UV/NH_3 and had λ_{max} (nm) 272, 332 (MeOH); 281, 304sh, 388 (NaOAc); 265sh, 277, 350, 384 (NaOAc/H_3BO_3) and 281, 330, 396 (NaOMe). Its R_f: (×100, Whatman No. 1, 30° C) 66 (H_2O), 50 (15% HOAc), 69 (30% HOAc), 75 (50% HOAc), 77 (60% HOAc), 32 (BAW), 63 (Phenol), 85 (Forestal) and 24 (t-BAW) was typical of flavone di-C-glycoside[4]. Compound B also yielded apigenin and glucose on HI fission and $FeCl_3$ oxidation respectively. The MS of the permethylether of B had signals at (m/z): 748 (M^+, 15%), 733 (M-15, 27%), 717 (M-31, 100%), 701 (M-47, 9%), 645 (M-103, 14%), 615 (M-133, 5%), 585 (M-163, 32%), 573 (M-175, 45%), 559 (M-189, 8%) and 543 (M-205, 6%) closely agreeing for apigenin 6,8-di-C-glucoside[11]. Identity of B was finally established as 6,8-di-C-β-D-glucopyranosyl apigenin (vicenin-2) by direct comparison with an authentic sample.

This is the first record of isolation of a flavone di-C-hexoside from the genus *Mollugo*. Other *Mollugo* sp. examined earlier contained mono C-pentoside[3,4], mono C-hexoside[3,6] and their 2″-O-hexosides[3,6] or di-C-pentoside[4] Vicenin-2 is a very rare flavonoid whose isolation from *M. hirta*, in comparison with the flavonoid pattern of other *Mollugo* species, has certain chemotaxonomic importance.

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A NEW METHOD FOR THE ESTIMATION OF ISONIAZID

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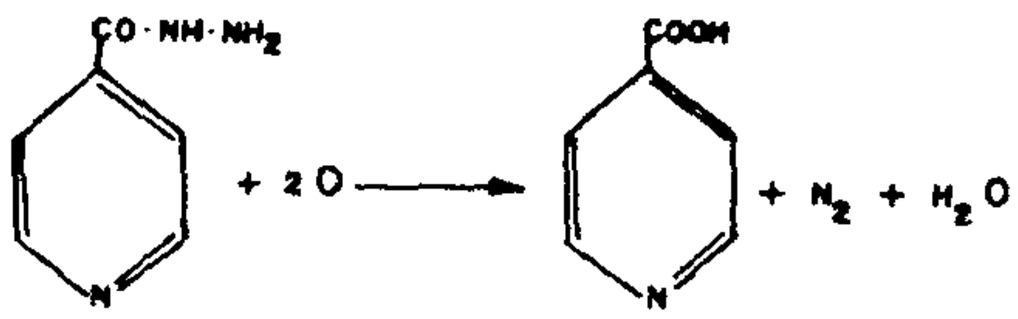
ISONIAZID, isonicotinohydrazide, is a highly effective tuberculostatic drug and many methods[1-7] are available for its determination. We report here yet another easy and reliable method using the reducing property of isoniazid. We have tried as oxidant, potassium permanganate, which oxidises isoniazid to isonicotinic acid. The oxidation of isoniazid by permanganate is somewhat slow at room temperature and the reaction is complete within 5 min at a temperature of 50–55°C. The equivalent weight of isoniazid for this reaction is established as molecular weight divided by four as shown below:



A definite quantity of the aqueous isoniazid solution is treated with a known excess of the $KMnO_4$ solution. The unreacted $KMnO_4$ is determined by adding pottassium iodide solution and titrating the liberated iodine against a standard sodium thiosulphate solution.

Isoniazid powder (0.75 g) is dissolved in 30–50 ml of water. In the case of the tablets, the solution is filtered to a 250 ml standard flask and made up by repeatedly washing with water. The isoniazid solution (20 ml) is pipetted into an iodine flask, 25 ml of 0.1 N $KMnO_4$ and 20 ml of 2N H_2SO_4 are added and heated for 5 min at 50-55° C and allowed to cool to room temperature. To this solution 20 ml of 10% KI is added and the liberated idodine is titrated against standard thiosulphate solution using starch as indicator (V_1). The end point is the discharge of blue colour. By a separate experiment the thiosulphate, eqvivalent of 25 ml of $KMnO_4$ solution (V) is determined.

$$\text{Percentage of isoniazid} = \frac{(V_2 - V_1) \times S_{thio} \times 34.27 \times 100}{20 \times 4 \times W}$$

where W = weight of isoniazid powder taken.

This method can be used to estimate pure isoniazid as well as ioniazid in various tablet forms such as Dosina. The error is found to be less than ±0.5%.

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COPPER INTERFERENCE IN NITRITE ESTIMATION BY DIAZOTIZATION AND ITS ELIMINATION

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NITRATE and nitrite are two important anions occurring in water, soil, vegetables and industrial waste whose concentrations need a careful evaluation. In many cases the concentration of nitrite is determined by a colorimetric procedure using a diazotization reaction[1,2]. This method is adapted for the determination of nitrate also after its reduction to nitrite. Automated systems have been developed for the rapid determination of these ions[3]. In such systems metallic wire reductors, treated with copper sulphate solutions have been employed to convert the nitrate into nitrite where contamination of the sample with copper is possible. Copper catalyses the decomposition of the diazonium salt and yields low results[2]. This was clearly observed when we attempted to determine the accumulation of nitrite in the culture medium of *Neurospora crassa* under conditions of copper toxicity[4].