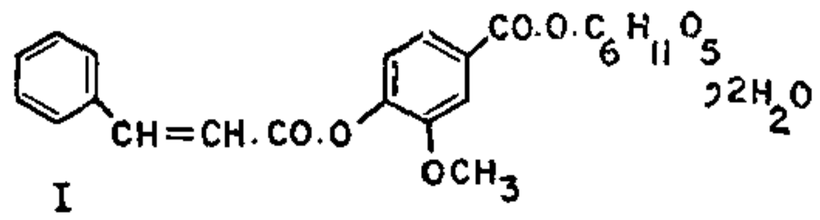
CHEMISTRY AND PHARMACOLOGY OF APOCYNIN, ISOLATED FROM *PICRORHIZA KURROA* ROYLE EX BENTH.

Picrorhiza kurroa Royle ex Benth. (Scrophulariaceae) (Hindi—*Kutki* or *Katki*)[1] is a wild plant which grows from Kashmir to Sikkim at an altitude of 9,000 to 15,000 ft. The roots and rhizomes are extremely bitter to taste and have been recommended as a substitute for the imported gentian roots. They are extensively used in the indigenous system of medicine as an antiperiodic, stomachic, cathartic, and cholagogue. In a detailed pharmacological investigation Das and Raina[2] observed that the water-soluble fraction of the alcoholic extract of the roots of *P. kurroa* possessed relaxant and non-specific antispasmodic effect on isolated ileum of rabbit, albino rat, rat uterus and intestine *in situ* of dog. Intravenous administration showed a prolonged choleretic effect in dogs and oral administration had laxative effect in albino rats. Daily oral administration in rats for one month protected the animals to a great extent against the carbon tetrachloride induced hepatic damage. These pharmacological observations along with the successful use[3] of the drug in clinical cases of infective and amoebic hepatitis indicated that the active principle of the drug is possibly kutkin, a bitter glucoside, $C_{23}H_{24}O_{10} \cdot 2H_2O$, m.p. 211°, $[\alpha]_D^{41} - 165°$, which was isolated and assigned structure (I) by Rastogi *et al.*[4]. In a recent communication[5] we have shown that structure (I) proposed for kutkin is not consistent with the biogenetic principles applicable to lignins, known to be derived from C_6–C_3 units and D-glucose precursors, and proposed a revised structure (II) for kutkin on the basis of chemical and physical evidences (i.r., n.m.r. and mass spectra). The free occurrence of vanillic acid in the roots of *P. kurroa*[4] prompted us to search for other lignin building stones, if any, present in the roots. We report here the isolation of 4-oxy-3-methoxy-acetophenone (III) from the roots of *P. kurroa* together with the pharmacology of the compound. This would further substantiate our contention for the new structure (II) proposed by us for kutkin on the basis of biogenetic considerations. The ketone (III) was first isolated under the name of *Apocynin* from *Apocynum canabinum*[6].

(*a*) *Chemical.*—The petroleum ether extract of dried milled roots of *P. kurroa* on fractional crystallisation afforded a crystalline ketone, $C_9H_{10}O_3$, m.p. 114–115° (needles from benzene, yield 0·033%); R_f 0·23 (SiO_2 G TLC; C_6H_6 : $CHCl_3$, 1 : 3; I_2 vapour); green colour with alcoholic $FeCl_3$, violet colour with aq. $FeCl_3$; positive Zimmerman reaction for $-CO.CH_2-$ group with *m*-dinitrobenzene and alkali; i.r. ν_{max} 3300 (OH), 1680 (CO) cm^{-1}; n.m.r. significant signals at δ 2·6 ($CH_3.CO.Ar$), 3·95 ($CH_3O.Ar$), 6·52 ($HO.Ar$), 6·92, 7·08, 7·35 ($H.Ar$) (Found: C, 65·2, 65·4; H, 6·13, 6·20. Calcd. for $C_9H_{10}O_3$: C, 65·05; H, 6·07). Mass spectrum showed the molecular ion peak at m/e 166 (M^+, 50%), and then significant fragment-ions at m/e 151 ($M-CH_3$, 100%), 136 ($M-2CH_3$, 5%), 123 ($M-COCH_3$, 18%), 108 ($M-COCH_3-CH_3$, 13%), 93 ($M-COCH_3-OCH_3+H$, 3%), 77 ($M-COCH_3-OCH_3-OH+2H$, 8%), 43 ($CH_3.C\equiv\overset{+}{O}$, 8%). The ketone formed a light yellow crystalline phenyl hydrazone, m.p. 125–126° (Found: C, 70·14, 70·28; H, 6·52, 7·02. Calcd. for $C_9H_{10}O_2=N.NH.C_6H_5$: C, 70·29; H, 6·29), and a colourless crystalline semicarbazone, m.p. 170–171° (alcohol-ether) (Found: C, 52·90, 53·40; H, 6·11, 6·47. Calcd. for $C_9H_{10}O_2=N.NH.CONH_2$: C, 53·80; H, 5·87).



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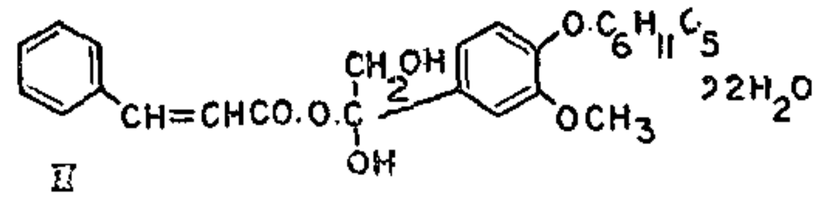

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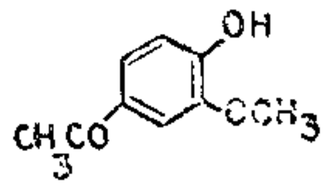

III

The ketone was identified as 4-oxy-3-methoxy-acetophenone (III) from a comparison of the above chemical and spectroscopic data with those of related isomeric compounds reported in literature, *viz.*, (*i*) 4-oxy-3-methoxy-acetophenone, apocynin, acetovanillone, m.p. 115°[6], 115–116°[7]; phenyl hydrazone, m.p. 125°[7]; semicarbazone, m.p. 167°[6], 172–173°[8]; (*ii*) 2-oxy-4-methoxy-acetophenone, paenol, m.p. 51°[9]; phenyl hydrazone, m.p. 108°[10]; (*iii*) 3-oxy-4-methoxy-acetophenone, isoacetovanillone, m.p. 66–69°[7], blue-violet colour with $FeCl_3$.

(*b*) *Pharmacological.*—An aqueous solution of apocynin, 4-oxy-3-methoxy-acetophenone, was used for pharmacological investigations, and the findings are as follows :—

1. *Smooth muscles.*—The effect of the drug was seen on isolated ileum of guineapig and rabbit, and isolated uterus of albino rat (quiescent and contracting). The drug produced a moderate to marked relaxation of rabbit ileum and contracting rat uterus in concentrations of 50–200 mcg/ml. It antagonised spasms induced by various spasmogens on guineapig ileum and rat uterus. The spasmolytic ED_{50} was calculated in each case by log dose-percentage inhibition curves and are given in Table I.

TABLE I

Spasmogen	Tissue	Spasmolytic ED_{50} of apocynin (mg/ml)
Acetylcholine bromide (0·01 mcg/ml)	Guineapig ileum	0·16
Histamine dihydrochloride (0·01 mcg/ml)	,,	0·21
Barium chloride (0·2 mg/ml)	,,	0·39
Oxytocin (0·01 U/ml)	Rat uterus	0·4
Serotonin creatine phosphate (0·01 mcg/ml)	,,	0·31

2. *Frog's rectus muscle.*—The drug had no effect on either acetylcholine or KCl induced spasms on this tissue, upto doses of 1 mg/ml.

3. *Perfused frog's heart.*—The drug produced a depressant effect on frog heart with reduction in force of contraction and heart rate in doses of 1–2 mg. The effect was a direct one since it was not blocked by pretreatment with atropine (10 mcg).

4. *Dog's blood pressure and respiration.*—The drug had no significant effect on anaesthetised (pentobarbitone sodium, 35 mg/kg I.P.) dog's blood pressure, heart rate and respiration upto doses of 5 mg/kg I.V.

5. *Biliary secretion in dog.*—In anaesthetised (pentobarbitone sodium, 35 mg/kg I.P.) dog with bile duct cannulated and cystic duct ligated, the drug produced a moderate increase in bile flow in doses of 5 mg/kg I.V. The volume increased twofold by one hour after drug administration and the effect persisted upto 180 min.

6. *Effect on general behaviour.*—The drug in doses of 50 to 100 mg/kg I.P. produced increased motor activity, piloerection, tremors, compulsive gnawing and salivation in albino rats.

SUMMARY

Apocynin, 4-oxy-methoxy-acetophenone, has been isolated from the petroleum ether extract of the roots of *Picrorhiza kurroa* Royle ex Benth., and its structure has been established by chemical and spectroscopic (i.r., n.m.r. and mass) evidences. Apocynin produces a moderate to marked relaxation of rabbit ileum and contracting rat uterus, and antagonises spasms induced by various spasmogens on guineapig ileum and rat uterus. It has a depressant action on frog's heart, and no significant effect on blood pressure. Apocynin possesses choleretic action. It produces a two-fold increase in bile flow in anaesthetised dog by one hour after drug administration and the effect persists upto 3 hours.

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OCCURRENCE OF OOCYTES IN THE KIDNEY OF THE CAT-FISH *HETEROPNEUSTES FOSSILIS* (BLOCH)

ALTHOUGH there are several reports on the occurrence of oocytes in the testes in fishes (*see* Atz)[1], instances citing the presence of oocytes in other tissues are rather scanty[2-4]. During the course of an investigation on the kidney of the cat-fish, *Heteropneustes fossilis*, the occurrence of oocytes in the kidney has been encountered in three instances and the present communication reports the same.