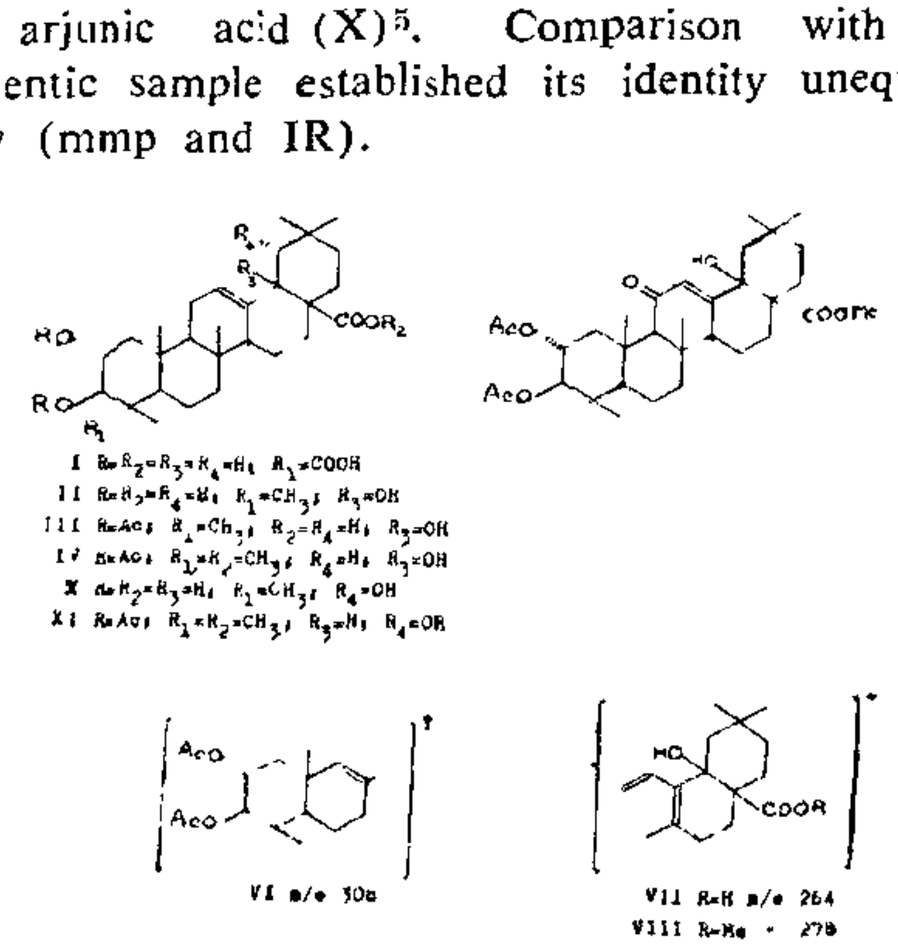
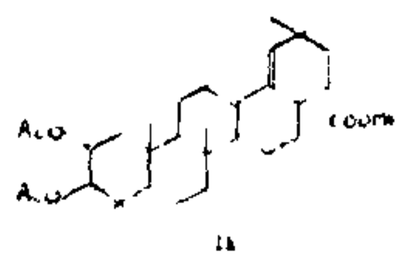
Mass spectral fragmentation[6] of diacetylacutangulic acid as well as its methyl ester furnished the expected RDA fragments (VI, VII and VIII) suggesting that the hindered hydroxyl might be in D or E ring and not in A or B. The location of this hydroxyl is revealed by its ready formation of methylanhydrodiacetylacutangulate (IX) m.p. 180–2°, $[\alpha]_D$ + 116° with $POCl_3$–Py. at reflux temperature or even glacial AcOH-sulphuric acid at reflux temperature for 4 hrs. This anhydro-ester (IX) contained a S *cis*-diene system (U.V. 246, 252 nm log ϵ 4·01 and 4·02 respectively), the new double bond conjugating with 12 : 13-double bond.

The NMR spectrum of the methylanhydro-diacetylacutangulate (IX) contained an additional proton at δ 5·43 d (J 1 Hz) in addition to the common 12 C–*H*, which appeared as a multiplet at δ 5·50. This negligible coupling constant for the new olefenic proton suggests a long-range coupling in the molecule. These experiments indicate that the tertiary hydroxyl could be located only at C–18. If it were so, the newly introduced double bond must occupy 18–19 positions and could be identical with methyl anhydro-diacetylarjunate, obtained by similar dehydration from methyldiacetyl arjunate (XI), a derivative of arjunic acid (X)[5]. Comparison with an authentic sample established its identity unequivocally (mmp and IR).





In an attempt to determine the steric configuration of the 18–hydroxyl, information was sought through lactonisation under normal conditions as in oleanolic acid[7]. Acutangulic acid (II) always afforded a mixture of compounds which could not be resolved. If the hydroxyl is 18 α-situated, lactonisation would be easier and a single product may be expected. Since a mixture is obtained it is presumed that D/E rings are *cis*- fused as in oleanolic acid and 18–OH is β-oriented.

On this basis acutangulic acid is tentatively assigned the structure 2 α, 3 β, 18 β-trihydroxy-olean-12 en-28-oic acid (II) and happens to be the first recorded instance of an 18 hydroxy penta-cyclic triterpene occurring in nature, although 20 α-hydroxy tetracyclic triterpenes and steroids (steroidal numbering) have been known for quite some time. Their origin must have been from a common non-classical carbonium ion intermediate during squalene cyclisation[8] which is believed to be responsible for the structural variation well known in rings D, E or the side-chain of triterpenes or steroids.

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OESTROGEN INJECTION TEST FOR EARLY PREGNANCY DIAGNOSIS IN MARES*

Review of Literature

TSUNEKANE *et al.* (1953) reported failure of heat symptoms in pregnant cows with subcutaneous injection of 100,000 i.u. of oestrogen one to three days before next expected oestrum. Nishikawa (1959) observed that with subcutaneous administration of 2·3 mg of stilbestrol diproprionate on 16th to 17th day

following last service, pregnant mares failed to return to oestrum; and non-pregnant showed oestrous symptoms within two to three days. The efficacy of the test was zero between two to twelve days, 50% on 13th day, 67% on 14th day, 86% on 15th day and 100% from 16th day onwards from last service. With combined administration of oestrogen and testostrone in sows the efficacy of the test claimed was 100% by Jochle and Schilling (1965) and 98% by Rothe (1971).

Materials and Methods

The present study was conducted at a thorough bred equine breeding station with 98 mares and six stallions. Thirty mares were selected at random for the study. Duration of oestrum of individual mares was recorded for a period of three months prior to the commencement of breeding season, by a study of their behavioural symptoms to teaser stallions or mares at paddocks, and by rectovaginal examination.

Two or three mg of stilbestrol diproprionate was injected subcutaneously on 16th to 17th day following last service and onset of oestrum investigated. Results recorded as pregnant were compared with those of rectal palpation and biological tests between 45 to 60 days.

False positive reactors were induced in oestrum by intrauterine infusion of about 1000 ml of normal saline with antibiotics at 37·5° C.

Results

Out of thirty mares tested 24% failed to exhibit oestrum following oestrogen injection during the first oestrum, 35% during the second, 18% during the third, 18% during the fourth and 6% during the fifth. The interval between the test injection and onset of oestrum was one day in 20%, two days in 20%, three days in 50% and six days in 10% mares. The efficacy of the test was 90%. Only three mares showed false positive reactions consistently throughout the breeding season.

Discussion

The oestrogen injection test in mares is based on the principle that the effect of administration of subclinical doses of oestrogen is related to the functional status of corpus luteum. Nishikawa (1959) reported that the incidence of induced oestrum in non-pregnant mares with administration of oestrogen varied from 0% between two to twelve days to 100% on 16th to 17th day after last service. In pregnant mares, on the other hand, the functional status of gravid corpus luteum remained undisturbed with the absence of oestrous symptoms. In the present study the efficacy of the test was 90% when compared with biological tests.

Repeated false reactions in three mares during the entire breeding season are probably due to early embryonic death with the resorption of foetus and persistence of corpus luteum. The incidence of early embryonic deaths in mares has been reported to be 10 to 30% by Britton (1947), and resorption of foetus resulting from corpus luteum insufficiency has been recorded between 42 to 100 days by Sagar (1962). Nishikawa (1959) observed false reactions in mares with prolonged oestrum and attributed them to persistency of corpus luteum.

False reaction in human patients to early pregnancy diagnosis with oestrogen-progesterone therapy has been attributed to persistency or cystic conditions of corpus luteum by Graber *et al.* (1970).

The fertility of the mares following the test dose of oestrogen remained unaffected. Development of normal follicles and incidence of 35% pregnancies following coitus during second, 18% each during third and fourth and 6% during fifth induced oestrums in the tested mares probably indicates absence of any adverse effects on ovarian activity. In equine breeding programmes this test seems to provide an efficient technique for confirmation of early pregnancy and for forced induction of a probable normal oestrum in non-pregnant mares thus providing repeated services in limited breeding season for obtaining highest conception rates.

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