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PRENYLATED COMPOUNDS FROM *ATALANTIA RACEMOSA*: ISOLATION AND SYNTHESIS OF TWO PYRANOFLAVONES

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Key Word Index—*Atalantia racemosa*, Rutaceae, racemoflavone, atalantoflavone; synthesis, 2D NMR spectroscopy, biogenesis; sonochemical methylation.

Abstract—A re-examination of the aerial parts of the plant *Atalantia racemosa* led to the isolation of two new pyranoflavones, namely atalantoflavone [8,8-dimethyl-5-hydroxy-2-(4′-hydroxyphenyl)-4H,8H-benzo-(1,2-*b*: 3, 4-*b*′) dipyran-4-one] and racemoflavone [8,8-dimethyl-5-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-4H,8H-benzo (1,2-*b*.3,4-*b*′) dipyran-4-one]. The structures have been confirmed by the synthesis of methyl ethers of atalantoflavone and racemoflavone. Besides the pyranoflavones, seven biogenetically related coumarin derivatives namely, xanthyletin, luvangetin, recemosin, xanthotoxin, umbelliferone, rutarin, rutaretin and a triterpene, friedelin have been isolated.

INTRODUCTION

Whilst screening plants for biological activities, the aerial parts of *Atalantia racemosa* (N. O Rutaceae) were found to possess insect antifeedant activity Therefore a detailed phytochemical investigation of *A racemosa* was undertaken. Joshi *et al* have reported the occurrence of xanthyletin (**1**), racemosin (**3**) and xanthotoxin (**4**) in this plant [1] In addition to these compounds, we have isolated friedelin, luvangetin (**2**), umbelliferone (**5**), rutarin (**7**) and rutaretin (**6**) Rutaretin and its glucoside rutarin, both of which contain the biogenetically significant α-hydroxyisopropyldihydrofuran moiety, have been isolated for the first time from this genus. The characterisation and synthesis of two new pyranoflavones for which we propose the names atalantoflavone (**8**) and racemoflavone (**11**) are described here [2].

RESULTS AND DISCUSSION

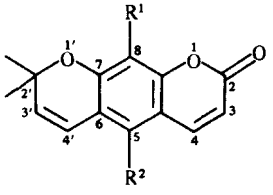
The petrol extract of the leaves of *A. racemosa* was found to contain a terpene, friedelin and four coumarins identified as xanthyletin (**1**), luvangetin (**2**), racemosin (**3**) and xanthotoxin (**4**) [1, 3] The linear structure **3** for racemosin as proposed by Joshi *et al.* [1] and Murray *et al* [4] has been further supported by NOESY experiment. The methanol extract gave three crystalline compounds which were identified as umbelliferone (**5**), rutarin (**7**) (major constituent) and rutaretin (**6**), the aglycon of rutarin (**7**). The co-occurence of umbelliferone (**5**), α-hydroxyisopropyldihydrofuranocoumarins (**6** and **7**), 2,2-dimethylpyranocoumarins (**1**–**3**) and furanocoumarin (**4**) presents interesting biogenetic inter-relationship [5].

The chloroform extract gave two new pyranoflavones which were designated as atalantoflavone (**8**) and racemoflavone (**11**)

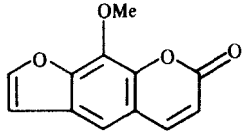
Atalantoflavone (**8**) has a molecular formula $C_{20}H_{16}O_5$ ($[M]^+$ *m/z* 336). Its flavonoid nature is indicated from the colour reactions and UV spectral data. It forms a diacetate (**9**) and dimethyl ether (**10**) indicating the presence of two hydroxyl groups. On the basis of the UV spectral data, the hydroxyl groups can be placed at positions 5 and 4′ [6]. The ^{1}H NMR spectra of **8** shows a singlet (6H) at δ 1.49 and two doublets (1H) at δ 5.78 and 6 94 (J = 10 Hz), suggesting the presence of 2,2-dimethylchromene moiety. Doublets (2H) at δ 7 01 and 8 01 (J = 9 6 Hz) can be assigned to protons at the 3′,5′ and 2′,6′ positions . A singlet at δ 6.70 is assigned to the proton at the 3 position [6]. Thus the remaining singlet at δ 6.19 must be due to the isolated proton on ring A. Based on these data two structures **8** and **13** are possible for atalantoflavone. The downfield shift of the aromatic proton on acetylation and methylation suggest the presence of the hydroxyl group adjacent to it. These results can only be explained on the basis of angular structure **8** [7]. Also, the alternate structure, **13**, has been assigned to caprochromene whose physical and chemical data are different from those of atalantoflavone [8].

Unequivocal proof to the structure **8** has been provided by synthesis of the dimethyl ether **10** of atalantoflavone. Isoevodionol (**14**), prepared by the known method [9], was anisoylated using anisoyl chloride–pyridine. The anisoyl ester **15** then underwent a Baker–Venkatraman transformation (KOH–pyridine) to the diketone **16** which was cyclized to compound **10** by PTS–benzene. The synthetic dimethyl ether was identical in all respects with the dimethyl ether obtained by ultrasonic irradiation of a mixture of atalantoflavone, Me_2SO_4 and K_2CO_3 in acetone (Scheme 1).

Racemoflavone (**11**) was obtained in very small amount (0.0007%). It has a molecular formula $C_{21}H_{18}O_6$, and its UV and IR spectra and colour reactions show close similarities with those of atalantoflavone. It forms the dimethyl ether **12** suggesting the presence of two hydroxyl groups The hydroxyl groups are placed at 5 and 4′ on the basis of the UV shift data [6]. The presence of a 2,2-dimethyl chromene moiety is indicated from the ^{1}H NMR data. A singlet (3H) at δ 4.00 indicated the presence of a methoxyl group. Doublets (1H) at δ 7 65 and 7.03 (J = 8 Hz) and a broad singlet (1H) at δ 7.61



1 $R^1 = R^2 = H$
2 $R^1 = OMe$, $R^2 = H$
3 $R^1 = R^2 = OMe$



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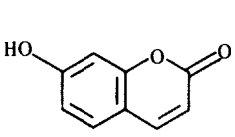

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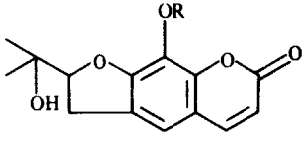
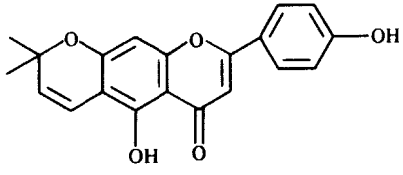


6 R = H
7 R = Glucosyl



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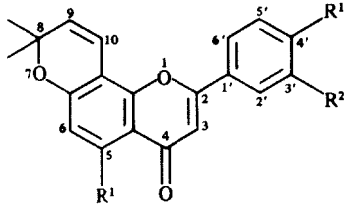
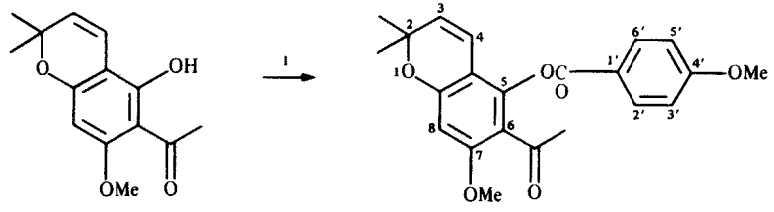
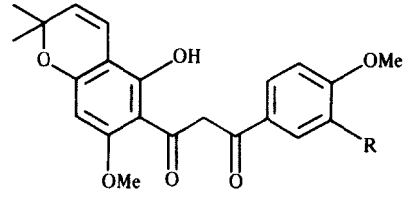


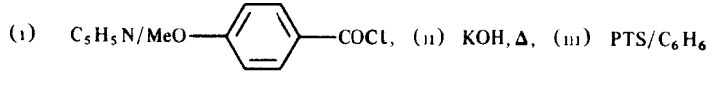
8 $R^1 = OH$, $R^2 = H$
9 $R^1 = OAc$, $R^2 = H$
10 $R^1 = OMe$, $R^2 = H$
11 $R^1 = OH$, $R^2 = OMe$
12 $R^1 = R^2 = OMe$

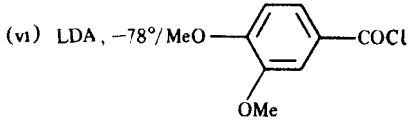


14 **15**



16 R = H
17 R = OMe





Scheme 1

suggest the presence of a 1',3',4'-trisubstituted ring B. A singlet at δ 6.74 can be assigned to proton H-3, while a singlet (1H) at δ 6.18 is assigned to H-6 [6, 7]. The chemical shift of H-6 shows the expected downfield shift on methylation On the basis of above data racemoflavone is assigned structure **11**.

The structure of racemoflavone was confirmed by synthesis. Attempts to synthesize racemoflavone using the Baker–Venkatraman method did not succeed. Therefore it was synthesized by an alternate route [10]. Isoevodionol (**14**), on lithiation with LDA at low temperature (−78°) formed an enolate, which on quenching with veratroyl chloride gave the diketone **17** Cyclisation of **17** with PTS–benzene gave compound **12**. The synthetic sample was identical in all respects with the dimethyl ether of racemoflavone (Scheme 1)

EXPERIMENTAL

Plant material Atalantia racemosa (N O Rutaceae) was collected from Mahabaleshwar (identified by Dr V Abraham and Dr S D Jaywant, Bhabha Atomic Research Centre) and a voucher specimen (No 1511) has been deposited in the herbarium of the Landscape and Cosmetic Maintenance Section, BARC Mps uncorr, UV MeOH, EIMS· Probe, 70 eV, ^{1}H NMR· 60 MHz and 500 MHz In the NOESY experiment the mixing time was 1 5 sec and 16 transients were accumulated for 250 values of evolution periods and a delay of 2 5 sec. was employed Chromatograph silica gel G Spots were detected under UV light (254 and 365 nm), by exposing the plates to I_2 vapour or by heating at 100° in an oven after spraying with 10% H_2SO_4 Acetate derivatives were prepared by the usual method (Ac_2O + pyridine overnight) Ultrasonic irradiations were carried out using a laboratory ultrasonic cleaner (40 KHz)

Extraction and fractionation Air-dried stems and leaves (1 4 kg) of *A racemosa* were powdered and extracted (Soxhlet) successively with petrol, $CHCl_3$ and MeOH The solvents were evapd and the residues were subjected to repeated CC employing solvent mixtures of increasing polarity. The petrol extract gave in order friedelin (310 mg), xanthyletin (560 mg), a mixture and xanthotoxin (480 mg) Separation of the mixture by repeated prep TLC (petrol–EtOAc, 3.1) yielded racemosin (800 mg, R_f 0 65, petrol–EtOAc, 7 3) and luvangetin (480 mg, R_f 0 63, petrol–EtOAc, 7:3) The MeOH extract on CC gave in order, umbelliferone (3 mg), rutaretin (40 mg) and rutarin (900 mg).

The $CHCl_3$ extract was put for CC over silica gel and eluted with petrol followed by $CHCl_3$ containing increasing amounts of MeOH Fractions eluting with $CHCl_3$–MeOH (49 1) gave a yellow solid which on prep TLC ($CHCl_3$–MeOH, 47 3) yielded racemoflavone (R_f 0.62) and atalantoflavone (R_f 0 33)

Atalantoflavone (**8**) Yellow needles from hexane–Me_2CO, mp 289–290°, (yield 45 mg, 0.003%) UV λ_{max}^{MeOH} nm (log ε) 233, 277, 312, 328 sh (4 51, 4 45, 4 42, 4 27), λ_{max}^{NaOMe} 229, 276, 394 $\lambda_{max}^{AlCl_3}$ 234, 280, 321, 352, 409, $\lambda_{max}^{AlCl_3+HCl}$ 234, 280, 320, 348, 409. IR ν_{max}^{KBr} cm^{-1} 3480 (OH), 2950, 2870, 1660 (CO), 1590, 1570, 1550, 1520, 1480, 1380, 1350, 840 ^{1}H NMR (Me_2CO-d_6, 500 MHz). δ 1 49 (6H, *s*, *gem* dimethyl), 5 78 (1H, *d*, *J* = 10 Hz, H-9), 6 19 (1H, *s*, H-6), 6 70 (1H, *s*, H-3), 6 93 (1H, *d*, *J* = 10 Hz, H-10), 7 01 (2H, *d*, *J* = 9 6 Hz, H-3', H-5'), 8 01 (2H, *d*, *J* = 9 6 Hz, H-2', H-6') MS *m/z* (rel int) 336 [M]$^+$ (19), 322 (21), 321 [M − Me]$^+$ (100), 308 [M − CO]$^+$ (3), 293 [M − MeCO]$^+$ (6), 203 (RDA of [M − Me]$^+$), 161 (6), 118 (10)

Atalantoflavone diacetate (**9**) Colourless needles from petrol–Me_2CO, mp 221–223° UV λ_{max}^{MeOH} nm (log ε) 232, 271, 328 (4 50, 4 42, 4 24) IR ν_{max}^{KBr} cm^{-1} 2980, 2930, 1765, 1750, 1645, 1600, 1580, 1500, 1480, 1380, 1340, 840 ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1 51 (*gem* dimethyl), 2 34 (3H, *s*, OAc), 2 42 (3H, *s*, OAc), 5 72 (1H, *d*, *J* = 10 Hz, H-9), 6 50 (1H, *s*, H-6), 6 56 (1H, *s*, H-3), 6 84 (1H, *d*, *J* = 10 Hz, H-10), 7 25 (2H, *d*, *J* = 8 3 Hz, H-3', H-5'), 7 86 (2H, *d*, *J* = 8 3 Hz, H-2', H-6')

Atalantoflavone dimethyl ether (**10**). A mixture of compound **8** (10 mg), K_2CO_3 (50 mg) and Me_2SO_4 (0 05 ml) in Me_2CO (5 ml) was irradiated with ultrasound at room temp till the completion of the reaction (2.5 hr) The reaction was worked up in the usual way (yield 8 mg, 80%) Pale yellow crystals from hexane–Me_2CO, mp 207–209°. UV λ_{max}^{MeOH} nm (log ε) 231, 275, 302, 348 sh (4 41, 4 44, 4 25, 4 02). IR ν_{max}^{KBr} cm^{-1} 2980, 2930, 2840, 1660, 1610, 1580, 1520, 1380, 1350, 1190, 1140, 1120, 840 ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1 50 (6H, *s*, *gem* dimethyl), 3 88 (3H, *s*, OMe), 3 95 (3H, *s*, OMe). 5 62 (1H, *d*, *J* = 10 Hz, H-9), 6 32 (1H, *s*, H-6), 6.58 (1H, *s*, H-3), 6 84 (1H, *d*, *J* = 10 Hz, H-10), 7 01 (2H, *d*, *J* = 8 5 Hz, H-3', H-5'), 7 81 (2H, *d*, *J* = 8 5 Hz, H-2', H-6') MS *m/z* (rel int.). 364 [M]$^+$ (66%), 350 (18%), 349 [M − Me]$^+$ (100), 334 (19%), 321 [M − MeCO]$^+$ (18%), 218 (RDA of [M − Me]$^+$ (94%), 203 (26%)

Synthesis of atalantoflavone dimethylether (**10**) (a) 6-*acetyl*-2,2-*dimethyl*-5-(4'-methoxybenzoyloxy)-2H-*benzopyran* (**15**) To a stirred soln of isoevodionol (**14**) (50 mg, 0 2 mM) in pyridine (3 ml), freshly dist anisoyl chloride (59 mg, 0.35 mM) was added After 30 min the reaction mixture was poured into ice-cold soln of 3% HCl (5 ml). The product was filtered, dried and crystallized from hexane–Me_2CO (yield 55 mg, 72%) Colourless crystals, mp 149–151° UV λ_{max}^{MeOH} nm (log ε) 216, 227, 257, 317 sh (4 40, 4 32, 4 59, 3 00) IR ν_{max}^{KBr} cm^{-1} 2980, 2950, 2860, 1735, 1690, 1645, 1610, 1580, 1515, 1475, 1370, 1345, 1160, 1100, 850 ^{1}H NMR ($CDCl_3$, 60 MHz): δ 1.43 (6H, *s*, *gem* dimethyl), 2 43 (3H, *s*, Ac), 3.80 (3H, *s*, OMe), 3 86 (3H, *s*, OMe), 5.46 (1H, *d*, *J* = 10 Hz, H-3), 6 26 (1H, *d*, *J* = 10 Hz, H-4), 6 33 (1H, *s*, H-8), 6 93 (2H, *d*, *J* = 8 Hz, H-3', H-5'), 8.0366 (2H, *d*, *J* = 8 Hz, H-2', H-6').

(b) 1,3-*Dioxo*-1-(5-*hydroxy*-7-*methoxy*-2,2-*dimethyl*-2H-1-*benzopyran*-6-*yl*)-3-(4'-*methoxyphenyl*)-*propane* (**16**) To a soln of compound **15** (40 mg, 0 10 mM) in pyridine (2 ml), pulverised KOH (50 mg) was added The mixture was stirred for about 30 min during which time a ppt of potassium salt was obtained The reaction was worked-up in the usual way The product **16** was crystallized from hexane–Me_2CO (yield 28 mg, 70%) Pale yellow crystals, mp 149–51° UV λ_{max}^{MeOH} nm (log ε) 221, 276, 308 sh, 381 (4 24, 4 54, 4 14, 4 10). IR ν_{max}^{KBr} cm^{-1} 3440, 2980, 2930, 2850, 1680, 1640, 1620, 1600, 1580, 1510, 1365, 1325, 1160, 1150, 1120, 840 ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1 46 (6H, *s*, *gem* dimethyl), 3 50 (3H, *s*, OMe), 3 90 (3H, *s*, OMe), 4.50 2H, *s*, −CO–CH_2–CO–), 5 44 (1H, *d*, *J* = 10 Hz, H-3), 5 76 (1H, *s*, H-8), 6 68 (1H, *d*, *J* = 10 Hz, H-4), 6.87 (2H, *d*, *J* = 8 Hz, H-3', H-5'), 8 03 (2H, *d*, *J* = 8 Hz, H-2', H-6'), 13 85 (1H, *s*, −OH chelated phenolic)

(c) *Atalantoflavone dimethyl ether* (**10**) Compound **16** (20 mg, 0 05 mM) in dry C_6H_6 (4 ml) containing dry *p*-toulenesulphonic acid (1 mg) was refluxed for 3 hr (monitored by TLC) The reaction mixture was washed successively with $NaHCO_3$ soln, H_2O and brine. The dried (Na_2SO_4) organic layer, on removal of solvent gave **10** which was purified by prep TLC (MeOH–$CHCl_3$ 1 19) It was crystallized from hexane–EtOAc as colourless needles. The synthetic dimethyl ether **10** was identical in all respects (TLC, mp, mmp, IR, UV, ^{1}H NMR and MS) with the natural dimethyl ether.

Racemoflavone (**11**) Yellow crystals from hexane–Me_2CO, mp 236–237°, (yield 10 mg, 0 0007%) UV λ_{max}^{MeOH} nm (log ε) 236, 274, 338 (4.13, 4 07, 3.90), λ_{max}^{NaOMe}. 216, 265, 409, $\lambda_{max}^{AlCl_3}$ 230, 280, 324 sh, 363, $\lambda_{max}^{AlCl_3+HCl}$ 229, 280, 324, 358 IR ν_{max}^{KBr} cm^{-1} 3460 (OH), 2940, 2860, 1665 (CO), 1580, 1560, 1520, 1480, 1380, 1350, 1200, 1135, 845 ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1 48, (6H, *s*, *gem* dimethyl), 4 00, (3H, *s*, OMe), 5 79 (1H, *d*, *J* = 10 Hz, H-9), 6 18

(1H, s, H-6), 6 74 (1H, s, H-3), 6 91 (1H, d, J = 10 Hz, H-10), 7 03 (1H, d, J = 8 Hz, H-5'), 7 61 (1H, br s, H-2'), 7 65 (1H, d, J = 8 Hz, H-6') MS m/z (rel int) 366 $[M]^+$ (21), 352 (20), 351 $[M-Me]^+$ (100), 338 $[M-CO]^+$ (15), 323 $[M-MeCO]^+$ (4), 203 (RDA of $[M-Me]^+$ (17), 175 (7), 148 (3)

Racemoflavone dimethyl ether (**12**) Methylation of racemoflavone was carried out in the same way as described for **8** Pale yellow crystals, mp 194–196° UV λ_{max}^{MeOH} nm (log ε) 238, 274, 329 (4.54, 4 48, 4 27) IR ν_{max}^{KBr} cm^{-1} 2940, 2880, 1660, 1610, 1580, 1525, 1370, 1355, 1140, 1130, 840 ^1H NMR ($CDCl_3$, 500 MHz) δ 1 52 (6H, s, *gem dimethyl*), 3 97 (6H, s 2xOMe), 3 98 (3H, s, OMe), 5 63 (1H, d, J = 10 Hz, H-9), 6.34 (1H, s, H-6), 6 60 (1H, s, H-3), 6 83 (1H, d, J = 10 Hz, H-10), 6 98 (1H, d, J = 8 5 Hz, H-5'), 7 32 (1H, br s, H-2'), 7 51 (1H, d, J = 8 5 Hz, H-6') MS m/z (rel int) 394 $[M]^+$ (66), 380 (29), 379 $[M-Me]^+$ (100), 364 (64), 351 $[M-MeCO]^+$ (3), 217 (RDA of $[M-Me]^+$ (58), 189 (4)

Synthesis of racemoflavone dimethyl ether (**12**) (a) 1,3-*Dioxo*-1-(5-*hydroxy*-7-*methoxy*-2,2-*dimethyl*-2H-1-*benzopyran*-6-*yl*)-3-(3',4'-*dimethoxyphenyl*)-*propane* (**17**) A mixture of isoevodionol (**14**) (50 mg, 0 2 mM) in THF (2 ml) and lithium diisopropylamide (0 5 mM, from diisopropylamine and *n*-butyllithium) in THF is stirred at −25° for 1 hr and then cooled to −78° and a soln of freshly dist veratroyl chloride (60 mg, 0 3 mM) in THF added The mixture, which turned yellow within 5 min, was stirred for 2 5 hr at −78° and then allowed to warm to room temp and set aside overnight It was then diluted with EtOAc (10 ml) and acidified with dilute HCl. The organic layer was dried (Na_2SO_4) and the solvent removed under red pres The residue obtained on purification by prep TLC ($CHCl_3$) gave compound **17** (yield 55 mg, 66 3%) Yellow crystals from hexane–Me_2CO, mp 179–181° UV λ_{max}^{MeOH} nm (log ε) 228, 275, 299, 385 (4 39, 4 56, 4 31, 4 08) IR ν_{max}^{KBr} cm^{-1} 3440 (*br*), 2990, 2950, 2850, 1690, 1650, 1625, 1610, 1580, 1520, 1370, 1335, 1160, 1150, 1125, 840 ^1H NMR ($CDCl_3$, 500 MHz) δ 1 43 (6H, s, *gem* dimethyl), 3 49 (3H, s, OMe), 3 95 (3H, s, OMe), 3.96 (3H, s, OMe), 4 51 (2H, s, $-CO-CH_2-CO-$), 5 45 (1H, d, J = 10 Hz, H-3), 5 80 (1H, s, H-8), 6 65 (1H, d, J = 10 Hz, H-4), 6 92 (1H, d, J = 8 Hz, H-5'), 7 55 (1H, br s, H-2') 7 56 (1H, d, J = 8 Hz, H-6'). 13 93 (1H, s, −OH chelated phenolic)

Racemoflavone dimethyl ether (**12**) Compound **17** (50 mg, 0 12 mM) in dry C_6H_6 (4 ml), containing PTS (1 mg) was refluxed for 3 5 hr (monitored by TLC) The reaction was worked-up as above Compound **12** was crystallized from hexane–Me_2CO (yield 38 mg, 76%) The synthetic dimethyl ether was identical in all respects (TLC, mp, mmp, IR, UV, ^1H NMR and MS) with the natural dimethyl ether **12**

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